Apoptosis in Human Disease: A New Skin for the Old Ceremony?

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Naturally occurring cell death or apoptosis is essential for the maintenance of tissue homeostasis and serves to remove extraneous or dangerous cells in a swift and unobtrusive manner. Recent studies have indicated a role for apoptosis in a plethora of human diseases. Hence, dysregulation of apoptosis has been implicated in autoimmune disease, acquired immune deficiency syndrome, and other viral (and bacterial) infections, as well as in neurodegenerative disorders and cancer. Furthermore, dysregulated apoptosis signaling may impinge on other age-related disorders such as osteoporosis and atherosclerosis and perhaps on the process of aging itself. The present review provides an overview of human diseases, which are associated with defective or inadvertent apoptosis, with examples of pathological conditions in which putative apoptosis defects have been elucidated at the molecular level. Novel apoptosis-modulating therapeutic strategies are also discussed.

Key Words: apoptosis; disease; pathogenesis; therapy.

Life and death are inextricably entwined. Glücksman (1), in his review of cell death in vertebrate ontogeny almost fifty years ago, cites numerous exam-

ples of naturally occurring cell death and emphasizes its role in the sculpting of tissues and organs. Similarly, Saunders (2), in his classical study on cell death in embryonic systems concludes that “abundant death, often cataclysmic in its onslaught, is a part of early development in many animals.” Yet, the study of cell proliferation and differentiation long prevailed over the study of cell death. In fact, naturally occurring cell death did not receive widespread recognition until the publication of the seminal paper on apoptosis by Kerr and colleagues (3). These authors highlighted the significance of controlled cell deletion and described the morphological features of this active and inherently programmed phenomenon, which they proposed plays a “complementary but opposite role to mitosis in the regulation of animal cell populations” (3). The importance of their study lies not only in the detailed morphological description of apoptosis (a term derived from the Greek word describing the falling off of petals from a flower or leaves from a tree), but also in the recognition of natural cell death as a wide-spread phenomenon not restricted to embryogenesis. An important corollary is that apoptosis is a genetically regulated process and as such may be amenable to therapeutic intervention. The aim of the present review is to discuss the potential involvement of apoptosis in human disease along with novel apoptosis-based therapeutic modalities now at hand.

THE ANATOMY OF CELL DEATH: APOPTOSIS VS NECROSIS

Apoptosis occurs in a well-choreographed sequence of morphological events (4). The cell first undergoes nuclear and cytoplasmic condensation with blebbing of the plasma membrane, a veritable dance macabre, which has been likened to “boiling” of the cytoplasm. Eventually, the cell breaks up into membrane-bound fragments termed apoptotic bodies containing structurally intact organelles, as well as portions of the nucleus. Subsequently, the apoptotic bodies are rapidly recognized, ingested and degraded by neighboring

Abbreviations used: AICD, activation-induced cell death; AIDS, acquired immune deficiency syndrome; AIF, apoptosis-inducing factor; ALPS, autoimmune lymphoproliferative syndrome; APAF, apoptotic protease activating factor; ARC, apoptosis repressor with caspase recruitment domain; BH, Bcl-2 homology; ced, cell death abnormal; eg, egg laying defective; FADD, Fas-associated death domain-containing protein; FLICE, FADD-like ICE; FLIP, FLICE-inhibitory protein; gld, generalized lymphoproliferative disorder; HIV, human immunodeficiency virus; IAP, inhibitor of apoptosis protein; ICE, interleukin-1β converting enzyme; IDDM, insulin-dependent diabetes mellitus; lpr, lymphoproliferation; MS, multiple sclerosis; NAIP, neuronal apoptosis inhibitory protein; NF-κB, nuclear factor κB; PS, presenilin; SLE, systemic lupus erythematosus; SMA; spinal muscular atrophy; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; zVAD-fmk, z-Val-Ala-Asp-fluoromethylketone.

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cells. Apoptosis is often remarkably inconspicuous histologically, and for this reason the phenomenon as such may have been neglected in earlier studies. Since apoptosis typically does not induce bystander cell death, inflammation or tissue scarring, it is well suited for a role in normal cell turnover during embryogenesis and in adult tissues (4). Necrosis, on the other hand, is a pathological or accidental mode of cell death, characterized by irreversible swelling of the cytoplasm and organelles, including the mitochondria. Eventually there is loss of membrane integrity resulting in cell lysis and release of noxious cellular constituents. Typically, a number of contiguous cells are affected, and exudative inflammation develops in the surrounding tissue. Necrosis occurs when cells are subjected to toxic stimuli such as hyperthermia, hypoxia, ischemia, complement attack, metabolic poisons and direct cell trauma (4). Moreover, under some pathological conditions both types of cell death, i.e., necrosis and apoptosis, may occur. For instance, ischemic damage is frequently characterized by a core of acutely damaged necrotic cells and a penumbra of cells which undergo delayed apoptotic death (5). The “decision” of the cell to die by necrosis or apoptosis is thought to depend largely on the severity of the insult (6, 7).

THE APOPTOSIS MACHINERY AND ITS REGULATION

The number of publications on apoptosis has grown exponentially over the last decade or so, due largely to the realization that apoptosis—the phenotype of cellular suicide—is an inherently gene-regulated process, the core components of which are conserved through evolution. The strongest evidence for an intrinsic death program comes from genetic studies in the nematode Caenorhabditis elegans (8). During postembryonic development of C. elegans, 131 of the 1090 cells die in every nematode. Loss-of-function mutations in two genes, ced-3 and ced-4, and gain-of-function mutations in a third gene, ced-9 (ced, cell death abnormal), were found to prevent all of these 131 naturally occurring deaths. The subsequent isolation and molecular characterization of these nematode “killer” genes revealed that ced-3, ced-4 and ced-9 all have mammalian counterparts known to be involved in apoptosis (for review see 8). Hence, ced-3 is highly homologous to the human interleukin-1β-converting enzyme, ICE (or caspase-1), while ced-9 was shown to be a functional homologue of the human anti-apoptotic protein Bcl-2. More recently, APAF-1 (apoptotic protease-activating factor-1) was demonstrated to be a human homologue of ced-4 (9) and additional mammalian ced-4 homologues have since then been unveiled (10). Collectively, these findings have underscored the remarkable degree of conservation of the cell death pathway from nematodes to humans, and are suggestive of the existence of a core death machinery in all cells.

A detailed account of the apoptosis signaling pathways employed in mammalian cells is beyond the scope of the present discussion, and has been subject to excellent reviews elsewhere (11, 12). However, it is important to note that the cytoplasmic cysteine proteases known as caspases, now comprising a family of 14 mammalian members, have emerged as the central executioners of apoptosis elicited by a variety of apoptotic stimuli (Fig. 1). In addition to their role in energy production, mitochondria have been also suggested to be critically involved in apoptosis signaling, with the early dissipation of mitochondrial transmembrane potential serving as a harbinger of death in numerous systems. Mitochondria are known to release apoptogenic factors upon apoptosis triggering, including the recently characterized AIF (apoptosis-inducing factor), cytochrome c and several pro-caspases, earning these organelles the epithet “poison cupboard” of the cell (13). The plasma membrane receptor designated Fas (also known as APO-1 or CD95) has emerged as a key regulator of apoptosis within the immune system. Fas-mediated apoptosis transpires via a caspase-dependent pathway, with mitochondria serving in some cases to amplify the initial death signal. The importance of Fas and its corresponding ligand in the homeostatic regulation of immune responses was underscored by the abnormalities observed in mice homozygous for the lpr and gld loci (14). As a result of mutations in the Fas and Fas ligand gene, respectively, these mice develop profound autoimmune disease, apparently due to the lack of apoptotic deletion of autoreactive lymphocytes. Apoptosis signaling instigated through death receptors such as Fas or directly via mitochondria is regulated, in turn, by the pro- and anti-apoptotic members of the rapidly expanding Bcl-2 family, which includes several human homologues of the recently characterized C. elegans death activator eg1-1 (8, 15). Finally, the swift recognition and engulfment of moribund cells by neighboring phagocytes is an important part of the apoptotic process (16). Indeed, apoptosis can be viewed essentially as a convenient cell clearance mechanism. Numerous candidate receptors and ligands have been identified which regulate phagocytosis of apoptotic cells in mammalian systems (17). In addition, genes regulating the clandestine clearance of dead cells in C. elegans have also been characterized and have in some cases proven to be homologues of known mammalian genes (8).

APOPTOSIS IN HUMAN DISEASE: TOO MUCH OR TOO LITTLE

In every human being about a hundred thousand cells are produced every second by mitosis, and a sim-
ilar number die by apoptosis (12). It is therefore of paramount importance that these events be tightly regulated. Consequently, the malfunction of the death machinery intrinsic to every cell may play a primary or secondary role in various diseases, with essentially too little or too much apoptosis (or apoptosis occurring in the wrong place and/or at the wrong time) leading to proliferative or degenerative diseases, respectively (Table 1). In the following sections some illustrative examples of diseases where apoptosis dysregulation has been implicated are highlighted, together with a brief discussion of the potential apoptosis-modulating therapeutic modalities prompted by these findings. Of note, evidence has now accrued for a role of apoptosis in most of the major human pathologies, including cancer, stroke and ischemic heart disease, and putative apoptotic mechanisms in disease should therefore not be merely of academic interest. However, the involvement of apoptosis in disease is not always straightforward, as demonstrated by the fact that certain viral infections trigger apoptosis while others appear to retard this process (Table 1). Moreover, autoimmunity may arise from a lack of apoptotic cell deletion as discussed earlier for the lpr and gld mice, although in some cases, unscheduled apoptotic destruction of cells may, instead, contribute to organ failure and autoimmunity. Finally, while cancer may be viewed simply as a result of ineffective apoptosis and hence a net gain of cells due to unrestrained proliferation, cancer cells themselves may evade immune surveillance by triggering apoptosis of patrolling immune cells. The following discussion aims to clarify these issues, and while in the process of doing so, perhaps give rise to some new and unanswered questions.

AUTOIMMUNE DISEASE

Systemic autoimmunity. The activation of T lymphocytes is known to lead to the concomitant upregulation of Fas and Fas ligand and to the susceptibility of these cells to Fas-mediated killing (so-called AICD, or activation-induced cell death) (18). AICD is essentially a mechanism for "switching off" the immune response and serves to limit the intensity of immune responses that might otherwise prove debilitating. It has been argued that chronic inflammatory disease, such as asthma, could result from an escape of activated T lymphocytes from Fas/Fas ligand-mediated cell death (19). Zhou et al. (20) showed that the protein kinase C inhibitor, bisindolylmaleimide VIII, markedly enhances the sensitivity of T lymphocytes to AICD. Treatment of animals with bisindolylmaleimide VIII in vivo abrogated autoantigen-specific immune responses as well as autoimmunity in two different models of T lymphocyte-mediated autoimmune disease. This indicates that accelerated induction of apoptosis in T lymphocytes can limit autoantigen-driven immune responses and thus offers a novel strategy for the treatment of autoimmune disease.

Studies conducted in lpr and gld mice have elucidated the role of the Fas system in peripheral selection and the maintenance of immune tolerance (14). Importantly, a syndrome in humans which closely resembles the murine phenotype has recently been described.
Two children were initially reported who displayed progressive lymphoproliferation associated with autoimmunity and large numbers of “double negative” T lymphocytes in the peripheral lymphoid system. Cellular and molecular studies of unrelated children with similar clinical findings revealed profound defects in lymphocyte apoptosis associated with mutations in Fas (21, 22). These children were grouped into a single entity designated autoimmune lymphoproliferative syndrome (ALPS). Additional reports have surfaced since the original characterization of ALPS, including studies of patients with Canale-Smith syndrome, which was interpreted as being identical to ALPS (23, 24). Moreover, two kindreds with a related, though more severe, phenotype were recently described (25). This syndrome, termed ALPS type II, is characterized by defective lymphocyte and dendritic cell apoptosis, yet with no molecular abnormalities in Fas or Fas ligand. Instead, these patients harbored mutations in caspase-10 resulting in impaired Fas-mediated apoptosis in lymphocytes as well as defective TRAIL-induced killing in both lymphocytes and dendritic cells.

Systemic lupus erythematosus (SLE) is a prototype systemic autoimmune disease, which is characterized by the presence of multiple autoantibodies and multiorgan immune destruction. A role for the Fas system in SLE was suggested by studies on soluble Fas (sFas), i.e., secreted Fas molecules derived from alternative splicing of Fas transcripts. Mice injected with sFas develop autoimmune symptoms and elevated serum

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<td>Diseases associated with increased apoptosis</td>
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<td>Hodgkin’s disease</td>
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<td>3. Autoimmune disorders</td>
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<td>Graft-versus-host disease</td>
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<td>Hashimoto’s thyroiditis</td>
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<td>Insulin-dependent diabetes mellitus</td>
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<td>Multiple sclerosis</td>
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<td>5. Toxic-induced disease</td>
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<td>Sepsis</td>
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<td>6. Bacterial and viral infection</td>
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<td>Shigella flexneri</td>
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<td>7. Miscellaneous</td>
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<td>Traumatic spinal cord injury</td>
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<td>Down’s syndrome</td>
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levels of sFas as were found in a number of SLE patients (26). Based on these findings, it was suggested that sFas may act as a ligand-binding decoy, thus preventing the apoptotic deletion of potentially autoreactive lymphocytes. Moreover, screening of DNA from 75 SLE patients has identified one individual with a heterozygous Fas ligand gene mutation with concomitant defects in Fas ligand function and AICD (27). Casciola-Rosen et al. (28) have shown that nuclear autoantigens, such as those targeted in SLE, are redistributed into cell surface blebs of apoptotic cells, and it was concluded that these surface blebs might serve as a stimulus for autoantibody production. Macrophage engulfment of apoptotic cells in vitro was decreased in SLE patients, and it was suggested that persistently circulating “apoptotic waste” (i.e., cells which are not effectively phagocytosed) may serve as immunogen for circulating “apoptotic waste” (i.e., cells which are not effectively phagocytosed) may serve as immunogen for the induction of autoreactive lymphocytes in vivo in these patients (29). In addition, the observation of increased numbers of uncleared apoptotic cells in the kidneys of the SLE-susceptible C1q-null mouse strengthens the idea that abnormal clearance of apoptotic cells may play a role in the pathogenesis of SLE (30). Taken together, these findings suggest that the defective clearance of apoptotic cells may represent a hitherto unrecognized role in the initiation and progression of autoimmune disease (16).

Organ-specific autoimmunity. In contrast to systemic autoimmune disorders that are characterized by B lymphocyte stimulation leading to antibody formation and autoimmunecomplex-mediated tissue injury, organ-specific diseases are characterized by T lymphocyte-mediated attack on specific cell types within the organ (31). Cell targets include the β-cells of the islets of Langerhans in insulin-dependent diabetes mellitus (IDDM), oligodendrocytes in the brain in multiple sclerosis (MS) and thyrocytes in Hashimoto’s thyroiditis. Recent data have implicated the Fas/Fas ligand system in the destructive phase of these autoimmune disorders (31). The first-described organ-specific autoimmune disease was Hashimoto’s thyroiditis (HT), yet attempts to define the mechanism responsible for thyrocyte destruction were unsuccessful for many years. Kotani et al. (32) provided evidence for apoptotic death in the thyroid gland of patients with HT. Thyrocytes from HT glands expressed Fas whereas non-autoimmune thyroids exhibited negligible amounts of Fas (33). On the other hand, Fas ligand was constitutively expressed in both normal and HT thyrocytes. It is likely that an autocrine self-destruction of thyrocytes, which coexpress Fas and Fas ligand, is a type of cell death analogous to AICD in T lymphocytes. Finally, recent data indicate that the Fas ligand-related TRAIL death pathway might also be operational in thyroid disease (34).

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system in which myelin and myelin-producing oligodendrocytes become the target of an inflammatory process resulting in plaque formation and neurological symptoms. Since destruction of oligodendrocytes results in the loss of myelin and a concomitant impairment of nerve conduction, considerable effort has been devoted to the identification of the mechanism(s) responsible for the death of these cells. In normal white matter, Fas expression is weak and scarce. However, analysis of acute and chronic MS plaques revealed a diffuse and intense Fas reactivity among the oligodendrocytes located along the lesion margin and in the adjacent white matter, along with the occurrence of Fas ligand-expressing microglioa and infiltrating lymphocytes (35). In addition, in vitro studies have shown that oligodendrocytes are susceptible to Fas-mediated killing (35), and that Fas governs astrocyte apoptotic responses in vitro (36), which might be of potential importance in MS. These observations are compatible with a role for Fas and Fas ligand in the destruction of oligodendrocytes in MS patients.

Insulin-dependent diabetes mellitus (IDDM) is a chronic autoimmune disease resulting from the destruction of insulin-producing β-cells in the pancreas. Much of what is known about the pathogenesis of IDDM comes from studies of the nonobese diabetic (NOD) mouse. Itoh et al. (37) showed that NOD mice bearing the lpr mutation were protected from the development of diabetes and adoptive transfer of splenocytes from diabetic NOD to these animals also failed to induce disease, suggesting that the Fas/Fas ligand system plays a key effector role in the pathogenesis of IDDM. On the other hand, Allison and Strasser (38) reported that Fas plays only a minor role in β-cell death in diabetic mice. Calcium is one of the most versatile and universal signaling molecules in the body and can function as either an inducer or inhibitor of apoptosis (39). We previously showed that specific calcium channel blockers prevent apoptosis in pancreatic β-cells treated with serum from patients with type I diabetes (40). Similarly, Srinivasan et al. (41) have shown that serum from type II diabetic patients with neuropathy induces calcium-dependent neuronal apoptosis. Moreover, high glucose concentrations and tolbutamid induce apoptosis in β-cells, which is dependent on intracellular calcium (42). Collectively, these findings have implications for the autoimmune destruction of insulin-producing β-cells evidenced in diabetes patients.

NEURODEGENERATIVE DISEASE

In the vertebrate nervous system up to 50% or more of different types of neurons die before embryonic development is complete. In other words, death gives birth to the nervous system. Only those neurons receiv-
ing enough neurotrophic support from their target cells will survive, and the rest are believed to be weeded out by the process of apoptosis (43). It has become apparent that excessive or inadvertent apoptosis also occurs in various pathological conditions in the brain (44). Particular interest has been devoted to the role of caspases in the pathogenesis of chronic neurodegenerative conditions such as Alzheimer’s disease and Huntington’s disease, although a role for caspases in acute traumatic neuronal injury has also been suggested (45). In addition, a promising caspase-based approach for the amelioration of nigral tissue transplant survival was recently reported (46). In this study, which may have important implications for the treatment of Parkinson’s disease, caspase inhibitors were shown to reduce apoptosis in embryonic nigral cell suspensions and to improve survival of dopaminergic neurons grafted to hemiparkinsonian rats, thereby improving functional recovery in these animals. The importance of caspases in neuronal apoptosis was further underscored by the restricted phenotype of caspase-3 and caspase-9 knockout animals, which displayed profound abnormalities in neural tissues despite a normal development of other organs including lung, liver and thymus (47).

Alzheimer’s disease. Alzheimer’s disease (AD) is the most common form of adult-onset dementia. AD is characterized by distinct neuropathological lesions including intracellular neurofibrillary tangle formation, extracellular deposition of β-amyloid, loss of synapses and neurodegeneration. The β-amyloid peptide has been shown to induce apoptosis in cultured neurons (48), and recent data indicate that the β-amyloid precursor protein may serve as a caspase substrate (49), thereby directly incriminating caspases in amyloidogenesis in AD. Genes encoding presenilin-1 (PS1) and presenilin-2 (PS2) were found to be mutated in most cases of early-onset familial Alzheimer’s disease (50). The cellular consequences of these mutations include an aberrant accumulation of β-amyloid peptide, derived from the β-amyloid precursor protein. Two apoptosis-promoting proteins, p53 and p21\textsuperscript{wat}, both inhibit PS1 production, suggesting that presenilins are normally anti-apoptotic. In fact, the presenilins are not only “switched off” during apoptosis, they can also undergo proteolytic processing associated with the activation of caspases (51). Such caspase-dependent cleavage results in loss of their anti-apoptotic function. Buxbaum et al. (52) have reported a novel calcium-binding protein, calseinilin, that binds both PS1 and PS2 and regulates the levels of their proteolytic products, thus providing a possible link between the presenilins, calcium homeostasis and caspase activation. Calcium presumably serves to induce conformational changes in calseinilin, with subsequent changes in the levels of anti-apoptotic presenilins. Moreover, ALG-3 (apoptosis-linked gene-3), a truncated PS2 cDNA encoding the calseinilin-binding region of PS2, protects against apoptosis possibly by sequestering calseinilin away from PS1 and PS2 (53). In sum, it is likely that β-amyloid peptide is the causative agent in Alzheimer’s disease, and PS mutations, along with activation of neuronal caspases, may act to increase the levels of this pathological peptide, thus leading to an increase in the degree of apoptotic destruction of neurons.

Spinal muscular atrophy. The childhood spinal muscular atrophies (SMAs), characterized by spinal cord motor neuron depletion, are among the most common autosomal recessive disorders. SMAs are subdivided as either type I, type II or type III, according to the age of onset and clinical severity, and the disease manifests as weakness and wasting of the voluntary muscles. Type I SMA is the most severe form and affected children rarely survive the first few years owing to respiratory muscle weakness. Many of the motor neurons observed at autopsy in the spinal cord of these patients are characterized by swelling and chromatolysis in a manner consistent with an apoptotic mode of cell death (54). The gene for neuronal apoptosis inhibitory protein (NAIP), a novel baculovirus IAP homologue, was recently mapped to the SMA region of chromosome 5q13.1 and found to be deleted in type I SMA individuals (55). These findings suggested that NAIP acts to promote neuronal survival, and that SMA might be caused by the pathological persistence or reactivation of normally occurring apoptosis in the spinal cord due to a lack of NAIP activity. Interestingly, the elevation of neuronal NAIP expression also confers resistance to ischemic damage in animal models of stroke (56). Several additional IAP homologues have been identified, including the four human IAPs, cIAP-1, cIAP-2, XIAP and survivin (57). The mechanism by which these novel proteins block cell death is largely unclear, but appears to depend, at least in some cases, on the direct inhibition of caspases-3 and -7 and of cytochrome c-induced activation of caspase-9 (58).

Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that occurs in both sporadic and familial forms. About 20% of the familial forms are associated with mutations in the gene encoding the cytosolic Cu/Zn superoxide dismutase (SOD) (59). Transgenic mice which express human familial ALS-associated mutant SOD develop a motor neuron degenerative syndrome that closely resembles ALS (60). Interestingly, the wild type Cu/Zn SOD acts as an anti-apoptotic gene in cultured neural cells, whereas the familial ALS-associated mutants act as dominant proapoptotic genes, despite the retention of significant SOD activity by some of the mutants (61). In situ hybridization studies revealed a decreased expression of Bcl-2 and a concomitant increase in Bax in ALS spinal cord motor neurons (62), suggesting that apoptotic mechanisms
Viruses have evolved exquisite strategies to inhibit host defenses. To circumvent these defenses, many virally infected cells via a Fas/Fas ligand-dependent mechanism. To prevent viral spread by recognizing and killing infected cells, cytotoxic T cells also eliminate viral DNA sequences which have been incorporated into the cellular genome. Cytotoxic T cells also recognize and kill cells expressing viral antigens.

In addition, apoptosis of an infected cell may occur by apoptosis in HD (65). Disruption of the HD gene in mice caused increased neuronal apoptosis and progressive behavioral and motor dysfunction (66). The HD gene product, huntingtin, is a substrate of caspase-3, or a closely related protease, and its cleavage was modulated by the length of the polyglutamine tract (67). Cytosolic aggregates of polyglutamine repeat proteins may recruit procaspase-8, most likely through binding of the adaptor protein FADD, resulting in the activation of caspase-8 (68). Taken together, these results suggest that caspase activation might play a role in HD, although it is presently unclear how the fragments liberated by caspase cleavage are linked to the demise of specific neuronal populations. Nevertheless, if the relationship between toxicity and caspase-mediated cleavage events can be formally demonstrated, then the administration of caspase inhibitors may, theoretically, serve to mitigate against cell death in these patients.

VIRAL AND BACTERIAL INFECTION

Viral disease. Two diametrically opposed hypotheses have emerged for the role of apoptosis in viral infections: (1) as a host antiviral defense mechanism, versus (2) as a pathogen-mediated mechanism to induce immune dysregulation and promote persistent infection. The validity of either of these hypotheses appears to depend on the specific viral infection examined (69). Hence, apoptosis of an infected cell may in some cases be viewed as a defense mechanism to prevent viral propagation, and the typical DNA fragmentation associated with apoptotic death may serve to eliminate viral DNA sequences which have been incorporated into the cellular genome. Cytotoxic T cells also act to prevent viral spread by recognizing and killing virally infected cells via a Fas/Fas ligand-dependent mechanism. To circumvent these host defenses, many viruses have evolved exquisite strategies to inhibit apoptosis of the infected cell. For example, establishment of an effective adenoviral infection depends on the function of the E1B 19K protein, a viral homologue of an anti-apoptotic protein Bcl-2 (70). Another adenovirus protein termed RID (receptor internalization and degradation) mediates internalization of cell surface Fas and subsequent destruction inside lysosomes within the cells, which may allow cells to resist Fas-mediated death and thus promote the survival of the virus (71). The p35 protein and the inhibitor of apoptosis proteins (IAPs) found in baculoviruses can inhibit apoptosis in response to a wide variety of stimuli (72), and cellular IAPs have subsequently been identified that are presumed to act as direct inhibitors of caspases. In addition, the cowpox serpin crmA has also been shown to inhibit the activation of caspases (73). Numerous herpesviruses, including Kaposi's sarcoma-associated herpesvirus (KSHV, also known as HHV-8) and herpesvirus saimiri, contain FLIPs (FLICE-inhibitory proteins) which possess sequences that interact with the Fas-associated adaptor protein, FADD (74). This FLIP-FADD interaction is thought to inhibit signaling events induced upon ligation of Fas or the TNF receptor by their respective ligands (74). Conversely, other viruses trigger apoptosis and/or cell lysis, perhaps for the purpose of facilitating viral release toward the end of the viral replicative cycle, and thereby succeed to promote viral infectivity and pathogenesis. A vivid example is provided by recent studies in victims of Ebola virus-infection, in which fatal outcome in patients was associated with massive intravascular apoptosis (75).

AIDS. Infection with human immunodeficiency virus is characterized by the gradual depletion of CD4+ T cells and the subsequent emergence of opportunistic infections and other manifestations of immunodeficiency which are collectively termed acquired immune deficiency syndrome (AIDS). It is therefore axiomatic that understanding the pathogenesis of HIV infection requires an understanding of the mechanisms involved in the loss of CD4+ T cells. A few years ago it was hypothesized that programmed cell death might account for the cell dysfunction and depletion in AIDS (76), and subsequent reports have substantiated the enhanced propensity of cells from HIV-infected individuals to undergo apoptosis. An increased susceptibility in both the CD4+ and CD8+ subpopulation of T cells has been described (77), and this appears in some cases to correlate with disease progression (78), although these findings have been disputed by others (79). Furthermore, it now seems apparent that during HIV disease, the battle between host and virus spills out into civilan territory, with the majority of T cell deaths in vivo occurring via apoptosis in uninfected "bystander" cells rather than in cells actually infected with the virus (80). Several mechanisms have been proposed to account for the enhanced degree of apoptosis induction.
evidenced in these patients, including apoptosis mediated by the HIV transactivator protein tat (81), AICD mediated by Fas/Fas ligand interactions (82), interaction of the viral envelope glycoprotein gp120 with CD4+ (83) or with the chemokine receptor CXCR4 (84) and cleavage of endogenous Bcl-2 by the HIV protease (85). However, apoptosis during the course of HIV infection may not always be detrimental to the host. The prevention of apoptosis in infected CD4+ T cells by adenovirus E1B 19K transfection resulted in enhanced viral production and the establishment of persistent high-level infection (86). More recently, Chinnaiyan et al. (87) arrived at similar conclusions, in a study aimed at the inhibition of caspases in HIV-infected cells, thus casting doubt on the feasibility of anti-apoptotic therapeutic modalities in HIV-infected individuals. On the other hand, a novel "Trojan horse" strategy to kill HIV-infected cells specifically was presented (88). By substituting proteolytic cleavage sites, these authors engineered an apoptosis-promoting caspase-3 protein that was processed into its active form by the HIV protease, resulting in apoptosis of the infected cell, but which remained inactive in uninfected cells. Future studies will show whether such an approach is fruitful in combating HIV infection.

Bacterial disease. An increasing number of bacteria have been identified as triggers of apoptosis in vitro (89). Activation of apoptosis has been proposed to serve as an efficient means for the bacterial "David" to strike the eukaryotic "Goliath" (89). For example, macrophages undergo apoptosis in vitro upon infection with Salmonella typhimurium, Shigella flexneri, and Yersinia pseudotuberculosis. It has been speculated that the induction of macrophage death may be important to initiate infection, promote bacterial survival and escape from host immune responses. Recent data indicate that LpaB, a bacterial invasin of S. flexneri, induces apoptosis by binding directly to caspase-1, a component of the macrophage apoptosis machinery (90). Since caspase-1 is also a pro-inflammatory molecule, induction of apoptosis may serve not only to delete host cells, but also to initiate inflammation. These findings raise the possibility of modulating the interaction between LpaB and caspase-1 as a target in both therapy and vaccine development. Moreover, a broad-spectrum caspase inhibitor, zVAD-fmk, prevented neuronal apoptosis and meningeal inflammation in an animal model of acute pneumococcal meningitis (91). The authors suggested that such caspase inhibitors, in conjunction with antibiotics, may provide a novel adjunctive treatment of bacterial meningitis and perhaps aid in the prevention of neurologic sequelae associated with this disease. It was reported recently that the obligate intracellular bacterial pathogen Chlamydia trachomatis, a leading cause of many important sexually transmitted diseases worldwide, possesses a novel anti-apoptosis mechanism, namely the blockade of mitochondrial cytochrome c release and subsequent caspase activation (92). Again, the elucidation of specific molecular targets of invading microbial agents may allow for more selective therapy of bacterial diseases.

The Neisseria porin protein PorB, a member of the large family of pore-forming proteins produced by gram-negative bacteria, is capable of translocating into membranes of infected target cells and functions in the infection process via an increase of cytosolic free calcium and subsequent activation of calpain as well as caspases (93). Intriguingly, PorB shares homology with the mitochondrial voltage-dependent anion channel (VDAC) and was therefore suggested to be a precursor of the permeability transition pore, a putative central regulator of apoptosis in mammalian cells. Helicobacter pylori infection is associated with chronic gastritis, peptic ulceration and gastric carcinoma (94). Helicobacter pylori triggers apoptosis in gastric epithelial cells and this process involves the activation of the Fas system (95). The involvement of Helicobacter pylori infection in the development of duodenal ulcers in vivo has been reported recently (96). It is possible that in the future, treatment protocols for the eradication of Helicobacter pylori infection may also include "anti-apoptotics" in addition to conventional antibiotics.

CANCER

Apoptosis and proliferation may be viewed as terms of the "growth equation" and too much growth, as in the case of cancer development, may result from too little death as well as from too much proliferation. Indeed, recent data have established a role for apoptosis dysregulation in all facets of cancer development, including hyperplasia, neoplastic transformation, tumor expansion, neovascularization and metastasis. Moreover, if cancer arises due to mutations that lead to the dysregulation of normal apoptotic death, then anti-neoplastic therapies designed to circumvent these intrinsic defects and specifically trigger apoptosis should prove beneficial. Novel apoptosis-based therapeutic tools would perhaps in the future be tailored specifically to the requirements of individual patients, depending on the profile of resistance and/or susceptibility toward apoptosis induction in ex vivo assays or on the profile of apoptosis-related genetic aberrations in silico (i.e., microarray-based) assays. The molecular basis for apoptosis dysregulation in cancer and cancer therapy will be reviewed in the following sections.

Oncogenes and tumor suppressor genes. Wyllie et al. (97) showed that cells which overexpress normal ras or myc protooncogenes induce tumors with high rates of both apoptosis and mitosis. However, cells that ex-
pressed the mutated, oncogenic form of the ras gene induced tumors with large numbers of mitotic cells, but few apoptotic cells. This was the first experimental evidence for the function of oncogenes as anti-apoptotic genes. Depending on the availability of critical growth factors expression of c-myc determines whether cells will undergo continuous proliferation or apoptosis (98). It is likely that c-myc does not itself induce apoptosis, but rather acts to sensitize cells to a wide range of distinct insults, such as serum or growth factor deprivation. For instance, c-myc was shown to act downstream of Fas through sensitization of cells to Fas-mediated killing (99). In addition, activation of c-myc induces sensitization to apoptosis through caspase-independent cytochrome c release from mitochondria, and the addition of survival factors blocks this release (100). Thus, the fate of a cell expressing c-myc is acutely dependent on both the availability of survival factors and on other signaling pathways, which may determine cell transition between growth arrest, proliferation and apoptosis.

Soon after the pioneering observations made by Wylie and colleagues (97), Vaux et al. (101) provided the first clues for the role of another oncogene in apoptosis. These authors found that bcl-2, the translocation of which results in the progression of high-grade lymphomas, extends pre-B cell survival and that it cooperates with the myc oncogene to immortalize lymphocyte precursors. Additional studies revealed that Bcl-2 contributes to tumorigenesis by extending cell survival through direct inhibition of apoptosis (102). Pathological elevations in the levels of one or more apoptosis-suppressing proteins of the Bcl-2 family (Bcl-2, Bcl-XL, Bcl-W, Mcl-1 and A1) have since been observed in several types of cancer. For example, high levels of Bcl-2 were observed in patients with B-cell chronic lymphocytic leukemia (B-CLL), although in this disease transcriptional activation rather than chromosomal abnormalities at the bcl-2 locus appear to be involved (103). Enhanced expression of Bcl-2 also appears to be common in neuroblastoma and is associated with unfavorable histology (104). However, in many tumors there may not be a direct correlation between overexpression of Bcl-2 and disease progression. For example, although small cell lung cancer (SCLC) cells expressed high level of Bcl-2 protein, these cells were more sensitive to spontaneous as well as radiation-induced apoptosis compared with non-small cell lung cancer (NSCLC) cells, which express low level of this protein (105). A decreased expression of Bax and Bak, pro-apoptotic members of the Bcl-2 family, has been reported in several human cancers (106). Moreover, experiments with knockout animals clearly demonstrated the ability of Bax to function as a tumor suppressor in vivo (107). In addition to transcriptional regulation of Bcl-2 family members, these molecules are also subject to post-transcriptional regulation (15). These mechanisms include the modification of proteins such as Bad via phosphorylation by Akt/Protein kinase B and other kinases. Bad is present as a doublet in many tumor cells, consistent with the presence of hyperphosphorylated Bad protein (108). Thus, high levels of phosphorylated Bad, along with other pro-apoptotic proteins from the same family, may conceivably be linked to resistance to therapy. On the other hand, it has been shown that phosphorylation of the anti-apoptotic proteins Bcl-2 and Bcl-X, leads to their inactivation and most likely to the sensitization of tumor cells to therapy (109). Furthermore, Bcl-2 can be cleaved by caspases in response to chemotherapeutic agents in vitro, resulting most likely in the loss of its anti-apoptotic function (110). Future studies will show whether cleavage of Bcl-2 plays a role in the sensitivity of tumor cells to treatment.

Mutations in p53 are the most common chromosomal aberrations in human cancer. The role of apoptosis in malignant disease was underscored by the observation that p53 can induce apoptosis (111) and that p53-dependent apoptosis can serve as a critical regulator of tumorigenesis (for review see 112). Although p53 is dispensable for normal development, experiments conducted in knockout mice confirmed that p53 acts as a tumor suppressor in vivo (113). Activation of p53 by DNA-damaging agents is critical for eliminating cells with damaged genomic DNA and underlies the apoptotic response of human cancers treated with ionizing radiation and DNA-damaging drugs (112). When cells suffer DNA damage, the p53 protein, also known as “the guardian of the genome” (114), accumulates due to post-translational stabilization and blocks entry into S phase. This transient delay at the G1 checkpoint in the cell cycle permits repair of damaged DNA prior to DNA replication. However, if DNA repair fails, p53 may trigger apoptosis. The expression of the p21<sup>WAF1</sup> gene is directly induced by p53, and the waf-1 protein has been implicated as an important effector of p53-mediated growth arrest (112). Using the serial analysis of gene expression (SAGE) technique, Polyak et al. (115) examined the transcripts induced by p53 expression, and found that many of the p53-induced genes (PIGs) encoded proteins related to the redox status of the cell. p53 is also known to upregulate the expression of the bax gene (116), thereby altering the apoptotic “rheostat” of the cell, i.e., the ratio of Bcl-2-to-Bax heterodimer formation. Another candidate effector molecule is the cell surface receptor Fas, which is transcriptionally upregulated by p53 (117). A recent report has also demonstrated that p53 can induce apoptosis through trafficking of Fas from intracellular stores to the cell surface (118). Moreover, APAF-1 and caspase-9 were recently found to serve as downstream effectors of p53-dependent apoptosis, and the disruption of these effector mechanisms of apoptosis facili-
Particular, TRAIL-induced killing could perhaps be exploited for the specific killing of tumor cells (131). In death receptor-triggered death, a finding that could be evidence that cells can protect themselves against tissues, but not in most cancer cells provided the first recovery of novel truncated TRAIL receptors in normal generated considerable interest (131). Moreover, the discovery of various chemotherapeutic agents may involve the Fas/Fas ligand system (121). Conversely, resistance against chemotherapy may conceivably arise due to mutations in components of the apoptotic machinery. A flurry of recent reports have described the occurrence of Fas mutations in patients with multiple myeloma (122), non-Hodgkin’s lymphoma (123) and adult T cell leukemia (124). In addition, recent studies have shown that resistance to chemotherapy is associated with resistance to Fas-mediated apoptosis (125), while others have provided evidence that Fas-independent pathways may also be involved (126). We have found that small cell lung cancer (SCLC) cells are lacking in pro-caspase-1, -4, -8, and -10 compared to non-small cell lung carcinomas (NSCLC), although the significance of these findings is presently unclear since SLCC cells are generally more sensitive to chemotherapy-triggered apoptosis (127). Similarly, the breast carcinoma cell line, MCF-7, carries a 47-bp deletion within exon 3 of the caspase-3 gene, although these cells remain susceptible to apoptosis triggering (128).

The Fas system may also play a role in the escape of tumor cells from immune surveillance. Hence, high constitutive Fas ligand expression has been demonstrated in different tumors and it was suggested that such tumors may eliminate activated Fas-expressing lymphocytes by induction of apoptosis (129). In addition, a novel Fas ligand decoy, DcR3 (decoy receptor 3) was recently identified, and suggested to enable tumor cells to evade surveillance by Fas ligand-expressing cytotoxic lymphocytes (130). In a sense, the tumor can be viewed as an immune privileged site (discussed below). Indeed, the combination of Fas resistance, due either to Fas mutations or secretion of decoy receptors, coupled with the ability of tumor cells to express Fas ligand effectively enables a preemptive strike or “counterattack” against the host immune system (129).

The recent finding that tumor cells are more sensitive to TRAIL-induced apoptosis than normal cells generated considerable interest (131). Moreover, the discovery of novel truncated TRAIL receptors in normal tissues, but not in most cancer cells provided the first evidence that cells can protect themselves against death receptor-triggered death, a finding that could be exploited for the specific killing of tumor cells (131). In particular, TRAIL-induced killing could perhaps provide the long sought-after means to trigger p53-independent apoptosis in tumor cells, which lack wild-type p53. Another finding, which sparked hopes of selective targeting of cancer cells, was the identification of a novel IAP homologue, designated survivin (132). Survivin was undetectable in terminally differentiated adult tissues, but abundantly expressed in transformed cell lines and in all common human cancers, suggesting that apoptosis inhibition may be a general feature of neoplasia. Similarly, the expression of several IAPs in human malignant glioma cell lines was suggested to play an important role in the resistance of these cells to apoptotic stimuli that directly target caspases (133). These findings may explain why cancer cells are impervious to signals that trigger apoptosis, and also offer an ideal target for apoptosis-based cancer therapy: if survivin or related IAPs are inactivated through therapeutic intervention then perhaps cancer cells can be made more susceptible to conventional cancer therapy. Finally, inhibition of the transcription factor NF-κB through adenoviral delivery of IκBα was recently shown to sensitize chemoresistant tumors to treatment resulting in tumor regression (134). These data suggest that inhibition of NF-κB and its associated anti-apoptotic activity, which may depend, in part, on the transcriptional activation of the survivin-related molecules cIAP1 and cIAP2, could be useful as an adjuvant therapy in cancer treatment.

Angiogenesis. Angiogenesis is the process leading to the formation of new blood vessels, and it has been proposed that tumor growth is critically dependent on such neovascularization. Hence, angiogenesis inhibitors are known to impair metastatic growth and sustain dormancy of tumors by indirectly increasing apoptosis (135). Cyclooxygenase-2 (COX-2), a key enzyme in regulation of eicosanoids, was shown to be inducible by a variety of apoptotic stimuli and to serve as an inducer of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), two regulators of angiogenesis (136). COX-2 also induces nitric oxide synthase (iNOS), which in turn is involved in the regulation of vascular permeability. Suppression of COX-2 activity and reconstitution of iNOS expression render tumor cells sensitive to chemotherapy treatment (137). Moreover, the combination of radiation therapy with angiostatin, a proteolytic fragment of plasminogen that inhibits angiogenesis, was shown to target tumor vasculature in vivo without an increase in toxicity toward normal tissues (138). Benjamin et al. (139) have recently demonstrated the selective vulnerability and death by apoptosis of immature tumor vessels following withdrawal of vascular endothelial growth factor (VEGF). Collectively, these findings indicate that neovascularization is the Achilles’ heel of tumors, and suggest that angiogenesis-based therapy might be an attainable goal also in the treatment of human cancer.
Atherosclerosis

Atherosclerosis is characterized by thickening of the arterial intima leading ultimately to cardiac and cerebral infarction (discussed below). Several cell types present in atherosclerotic lesions, including endothelial cells, smooth muscle cells and macrophages, are known to undergo apoptosis with varying outcome. For instance, apoptotic death of endothelial cells has been suggested to be an initial step in the development of atherosclerosis (for review see 140). This hypothesis is supported by the observation that pro-atherosclerotic factors such as angiotensin II, oxidized low density lipoprotein, reactive oxygen species and inflammatory cytokines all induce apoptosis in endothelial cell, whereas known protective factors such as estrogen and nitric oxide prevent apoptosis in these cells. Furthermore, apoptosis is involved in the turnover of lipid-laden foam cells of both macrophage and smooth muscle cell lineage in atherosclerotic lesions in murine models of atherosclerosis (141). Cai et al. (142) have reported evidence that Fas may regulate apoptosis of foam cells in advanced human atherosclerotic lesions. Animal studies show that activation of the nitric oxide synthase induces apoptosis of vascular macrophages in intimal lesions, and this was suggested to play a role in the regression of atherosclerosis (143).

Loss of smooth muscle cells in vulnerable regions of an atherosclerotic plaque may jeopardize the integrity and stability of the plaque. Importantly, apoptosis of vascular smooth muscle cells has been demonstrated in atherosclerotic plaques (144). Furthermore, it is likely that apoptotic vascular smooth muscle cells can promote thrombosis directly by exposure of phosphatidylserine on the cell surface (144). Mallat et al. (145) observed high levels of shed membrane microparticles (apoptotic bodies) with procoagulant potential in human atherosclerotic plaques, suggesting a role for apoptosis in plaque thrombogenicity. We found that human aortic smooth muscle cells treated with 25-hydroxycholesterol, an oxidation product of cholesterol, underwent apoptosis with activation of caspase-3 (146). Apoptosis was potentiated by TNFα and IFNγ, suggesting that the effects of lipid oxidation products on cells in atherosclerotic lesions may be regulated by inflammatory cytokines present in the microenvironment. Furthermore, apoptosis was inhibited by the calcium channel blockers verapamil and nifedipine, thus serving to underscore the role of calcium as a second messenger in apoptosis signaling.

Accelerated atherosclerosis of the coronary arteries following chronic rejection is the most common cause of heart transplant failure. Recent studies have indicated a role for anti-apoptotic genes, including Bcl-XL, in the prevention of chronic cardiac graft rejection (147). In addition, induction of heme oxygenase-1 (HO-1), the rate-limiting enzyme in the degradation of heme, also protected allografts against chronic injury and transplant atherosclerosis. These data concur with previous findings in mice with a targeted disruption of HO-1, in which prolonged survival of cardiac xenografts could not be achieved (148). More recently, HO-1 was shown to afford protection against apoptosis by virtue of its augmentation of iron efflux from cells (149). These findings may allow for the development of new strategies to control graft rejection.

STROKE AND CARDIAC DISEASE

Stroke. Neuronal death in stroke has previously been attributed to necrosis. However, numerous studies have indicated that apoptosis may also contribute to neuronal demise in oxygen-deprived brains (44). Moreover, several studies conducted in animal models have implicated caspases in neuronal cell death after stroke. Hara et al. (150) showed that intraventricular injection of caspase-1 inhibitors can reduce ischemic neuronal loss (although, considering the role of caspase-1 in cytokine processing and inflammation, the reduction in cell death evidenced in these studies may perhaps reflect an inhibition of inflammatory rather than apoptotic damage). More recently, systemic or intraventricular injection of a pan-caspase inhibitor or a caspase-3-selective caspase inhibitor afforded neuroprotection in animal models of neonatal hypoxic-ischemia (151) and focal ischemic infarction (152). Caspase-3 activity was also shown to increase after experimental cerebral ischemia in rodents (153) and XIAP overexpression prevented the activation of caspase-3 under these conditions, thus allowing neurons to survive after the ischemic insult (154). Furthermore, a breach in the mitochondrial barrier and the release of mitochondrial caspase-9 was demonstrated in an animal model of cerebral ischemia, suggesting that mitochondrial events may be central in post-ischemic neuronal damage (155). Suppression of apoptosis may prove particularly useful in acute disease processes such as stroke and we anticipate a rapid translation of anti-apoptotic therapies from the laboratory to the clinic. Indeed, drugs that inhibit apoptosis may be effective in the prevention of reperfusion-induced injury (discussed below) even when administered some time after the stroke or myocardial infarction has occurred.

Ischemia/reperfusion. Ischemia, i.e., oxygen starvation, of tissues during arterial occlusion, shock and organ transplantation is a common and important cause of death (for review see 156). Following the initial insult, cells may die rapidly by necrosis as a result of hypoxia and decline of energy levels. Additional loss of parenchymal cells by apoptosis may occur during reperfusion of the affected organ (156). Fas/Fas ligand-dependent mechanisms have been proposed to account at least for some forms of reoxygenation-induced apop-
tosis. Rasper et al. (157) reported that the caspase inhibitor usurpin (also known as FLIP) was abundantly expressed in cardiac and skeletal muscle and was downregulated in cardiac tissue following ischemia/reperfusion injury in vivo. The distribution of usurpin was the reciprocal of that of active caspase-3, which was abundant in apoptotic, infarcted tissue and low in unaffected regions of the heart. Usurpin may therefore act as a modulator of apoptosis sensitivity in cardiac myocytes following ischemia/reperfusion injury. ARC (apoptosis repressor with caspase recruitment domain) is another apoptosis-regulating molecule expressed exclusively in skeletal and cardiac muscle (158). ARC was shown to selectively target caspases, and delivery of ARC by gene transfer or enhancement of its endogenous activity may serve as a novel strategy for treatment of diseases with excessive muscle cell apoptosis.

Heart failure. Evidence for a role of caspases in cardiac development comes from recent knockout studies in mice (for review see 47). Deletion of the mouse caspase-8 gene resulted in impaired heart muscle development and death in utero, presumably from cardiac failure. Similarly, gene targeting of the caspase adaptor protein, FADD, resulted in a lethal phenotype with profound signs of cardiac failure and hemorrhage. Apoptosis may also play a role in cardiac disease such as congestive heart failure, a major cause of cardiovascular hospitalization. Congestive heart failure is defined as the inability of the heart to provide adequate blood flow, oxygen, and nutrients to tissues and organs (159). Regardless of the origin of the cardiac insult leading to congestive heart failure, such as myocardial infarction, ischemic cardiomyopathy and hypertension, the progression of heart failure is invariably manifested as cardiac remodeling with loss of left ventricular mass. The mechanisms responsible for the thinning of ventricular tissue are poorly understood, but may, at least in part, occur by apoptosis of cardiac myocytes. For instance, evidence was recorded for apoptotic myocyte loss in cardiomyopathy associated with certain arrhythmias and in ischemic and dilated cardiomyopathies of end-stage heart failure (159). Moreover, apoptosis of myocytes in patients with intractable congestive heart failure was demonstrated despite the enhanced expression of Bcl-2 in these cells (160). Cardiac myocytes and their skeletal muscle counterparts are characterized by an unusually high density of mitochondria, and we recently showed that skeletal muscle cells apparently do not express APAF-1, the protein "platform" required for post-mitochondrial caspase activation (161). By contrast, Narula et al. (162) provided evidence for mitochondrial cytochrome c release and activation of caspase-3 in end-stage human cardiomyopathy, suggesting a molecular basis for novel interventional strategies in conditions with myocardial dysfunction.

TRANSPLANTATION

Graft rejection is a potential threat in all cases of tissue transplantation. However, there are certain immune privileged sites in the body, such as the testis and the anterior chamber of the eye, where allogeneic or xenogeneic tissue grafts enjoy prolonged survival relative to other areas. While immune privilege was originally perceived as a passive process relying on physical barriers and isolation, recent data support the view of immune privilege as an active phenomenon. Specifically, a number of recent papers have indicated a role for Fas in maintaining the immune privileged state and implicated the Fas system in both the regulation of physiological cell turnover and the protection of particular tissues against lymphocyte-mediated damage (163). Thus, testicular allografts expressing functional Fas ligand evaded rejection, presumably by inducing apoptotic cell death of Fas expressing recipient T cells activated in response to graft antigens (164). Similarly, interactions between Fas and its ligand are important for the maintenance of immune privilege in the eye (165). A recent report described the prevention of rejection of insulin-producing islet cells in diabetic mice by cotransplantation of syngeneic myoblasts genetically engineered to express Fas ligand (166). While some of these findings have been disputed by other investigators (167) they nevertheless provide a rational for the manipulation of the local environment of a graft in a highly site- and immune-specific manner, and could represent the beginning of a new era in transplantation immunology. For instance, it may be feasible to endow allografts or xenografts with the property of immune privilege, or resistance to rejection, by expressing Fas ligand (and possibly other death ligands such as TRAIL) in the donor tissues, as outlined above. Indeed, the development of a "universal" graft which any recipient could accept, such that the tissue would not be destroyed in a transplant rejection, would be paramount to attaining "the Holy Grail of transplant biology" (168).

AGING

The process of aging should be distinguished from the diseases of aging such as cancer: the phenotype of aging affects all individuals in a population while diseases of aging affect only a subset (169). Several studies have shown that single gene mutations may significantly extend the life-span of fruit flies and nematodes (for review see 169). For instance, age-1 mutants of C. elegans have increased levels of SOD and catalase, are more resistant to oxidative stress and live twice as long as wild-type nematodes. Similarly, the long-lived Dro-
sophila mutant methuselah is resistant to oxidative stress as well as starvation. In humans, on the other hand, heritability of life-span is relatively minor (170). Nevertheless, several alternative molecular models of aging exist which may be applied to the human species. The “free radical” theory of aging, for instance, states that reactive oxygen species, which are generated by cellular metabolism, cause cumulative damage over a lifetime. Genome instability, i.e., the accumulation of genomic changes over time, has also been suggested as a cause of aging. Much attention has focused on the reactivation of the telomerase enzyme in cultured human cells, which can extend the replicative life-span of these cells beyond the normal limit (171). However, it remains to be tested whether telomere shortening and cellular senescence are causally related to aging. Finally, dysregulated apoptosis may contribute to aging, although genetic studies in C. elegans appear to argue against a relationship between programmed cell death and senescence, at least in nematodes (172). Mitochondria are of particular interest in this context, given the evidence for the role of these organelles in apoptosis (173). Mitochondrial function apparently deteriorates during normal aging, as evidenced by a decline in electron transport, accumulation of mitochondrial mutations, and decreased bioenergetic capacity (measured by mitochondrial membrane potential) (174). Hence, an accumulation of mitochondrial DNA mutations has been observed in aging as well as in cancer and is due, most likely, to oxidative damage, which increases with age. Cells bearing pathogenic mitochondrial mutations are sensitized to oxidative stress, which in turn is known to induce permeability transition, a putative central regulator of apoptosis (174).

Down’s syndrome serves as an example of premature or pathological aging (175). These individuals have a decreased life expectancy and display many progeroid features, including neurohistopathological changes similar to those found in Alzheimer’s disease (i.e., senile plaques and neurofibrillary tangles). An in vitro study on Down’s syndrome patient cells provided evidence for neuronal apoptosis, which was caused by a defect in the metabolism of reactive oxygen species (176). To conclude, (systemic) aging may perhaps be viewed as a mitochondrial disease associated with oxidative stress and dysregulated (cellular) apoptosis. On the other hand, the inability of cells to undergo apoptosis may also be related to aging and more specifically to the diseases of aging, since senescent, precancerous cells may accumulate under such conditions. By this interpretation, “successful aging” might depend on the maintenance of apoptotic mechanisms to eliminate cells that have sustained DNA damage (175). Further studies are needed before the role of apoptosis in aging is understood.

**APOPTOSIS AS A THERAPEUTIC TARGET**

Does apoptosis occur in human disease? While evidence for the involvement of dysregulation of apoptosis appears readily forthcoming in some cases (for instance, defective apoptosis in ALPS due to inherited Fas mutations) in other cases, such evidence is merely circumstantial, and often based on crude methods of apoptosis detection in postmortem clinical specimens. An illustrative example is provided by recent observations in Creutzfeld-Jacob's disease, a neurodegenerative condition characterized by the accumulation of a post-transcriptionally modified pathological form of the host-encoded prion protein. A prion protein fragment was shown to induce apoptotic cell death in vitro in primary rat hippocampal neurons (177) and the normal cellular isoform of the prion protein serves to protect against apoptosis in vivo in an animal model of prion disease (178). However, Ferrer (179), in a study of human brain specimens from deceased patients using in situ end labeling of fragmented DNA, has shown that none of the positive cells displayed apoptotic morphology. Moreover, deposits of prion protein were not associated with increased staining of fragmented DNA. These findings suggest that the mere presence of DNA fragmentation in clinical specimens does not necessarily indicate apoptosis, a conclusion supported by other investigators in the field (180). Indeed, while caspase activation may be viewed as an integral feature of apoptotic cell death (181), even the detection of activated caspases or cleavage of typical caspase substrates may not suffice to label cells as "apoptotic." For example, caspase activation in heart muscle cells may occur despite the conspicuous absence of other indices of apoptosis (162). In addition, caspase activation and cleavage of the classical caspase substrate, poly(ADP-ribose) polymerase, has been demonstrated in activated, non-apoptotic T lymphocytes (182). Therefore, great care should be taken to assess apoptosis by both morphological and biochemical criteria and attempts should also be made to devise improved methods for the detection of apoptosis in vivo and in fixed pathological specimens.

Does apoptosis play a role in disease pathogenesis? The view that disease can result from the dysregulation of active cellular suicide mechanisms represents a novel paradigm in medicine. A critical question, therefore, is whether the labeling of dead cells as "apoptotic" has added to our understanding of disease processes, or whether this is merely, in the words of the Canadian singer/songwriter Leonard Cohen, a new skin for the old ceremony? Indeed, to establish that apoptosis occurs (or is lacking) in a particular disease does not mean to say that apoptosis defects are directly implicated in the pathogenesis of the condition and therefore a legitimate target for therapeutic intervention, i.e., involvement of apoptosis cannot a priori be
equated with causality (183). For instance, it could be argued that the primary cause of T cell depletion in AIDS is not apoptosis per se but rather the inability of lymphopoiesis to replace the CD4+ T cells lost prematurely because of the infection with HIV. In this case, the enhanced rate of T cell apoptosis in cells obtained from HIV-infected persons may merely be reflective of a chronic immune activation in these individuals, rather than being involved in the pathogenesis of the disease itself. Similarly, the recent finding that cells from patients with Niemann–Pick’s disease are resistant to radiation-induced apoptosis due to an inherited deficiency in acid sphingomyelinase activity (184) does not necessarily imply that these aberrations are associated with the development or progression of disease. Therefore, the present challenge lies in the identification of those conditions in which apoptosis plays a causal role, that is, those diseases in which cellular self-annihilation is linked to the pathogenesis or progression of disease and not merely a consequence of underlying mechanisms.

Can apoptosis be safely targeted for therapy? An important question is how to harness apoptotic processes for therapeutic benefit while maintaining an adequate degree of apoptosis in unaffected “bystander” tissues. Indiscriminate inhibition of apoptosis could lead to the survival of genetically damaged cells which would otherwise die, thus causing widespread hyperplasia, whereas inappropriate promotion of apoptosis might lead to undesirable tissue degeneration (185). Indeed, Wickremasinghe and Hoffbrand (186) caution that anti-cancer strategies dependent solely on the induction of apoptosis may result in the rapid evolution of clones resistant to killing. Nevertheless, one may envisage a number of principle categories of apoptosis-regulating therapies (185, Fig. 2), which may be utilized alone or in conjunction with conventional treatments: (a) injectable molecules targeted at upstream modulators of apoptosis, such as death receptor decoys or soluble death ligands; (b) small molecule pharmaceuticals designed to regulate expression or activation of apoptosis-related genes and gene products, including Bcl-2, caspase family members and p53; and (c) gene therapy, e.g., overexpression of pro-apoptotic Bcl-2 family members or replacement of p53. The first clinical results of p53 reconstitution by gene therapy were reported by Roth et al. (187), who obtained tumor regression in three patients and tumor stabilization in three others out of nine non-small cell lung cancer-afflicted individuals with p53 mutations. Utilizing adenoviral vectors as an alternative delivery system, Bischoff et al. (188) were able to induce complete regression in 60% of implanted tumors in nude mice bearing human cervical carcinoma tumors. These authors used an E1B 55 kDa-deficient adenovirus which is only able to replicate in and kill p53-deficient cells, and these results therefore demonstrate the feasibility of using anti-tumor agents that kill only p53-deficient tumor cells, leaving normal cells unaffected. Future work should aim at the design of therapies specifically targeted against components of the apoptosis pathway in diseased tissues while leaving bystander tissues unscathed.

Does prevention of apoptosis restore function? Another important question to consider is whether cells rescued from apoptosis are functional or whether they are simply “undead” or anergic and, as a result of inadequate expression of recognition signals, not readily available for phagocytic clearance. In other words, rescuing a cell from death may not necessarily be the same thing as preserving its function. Indeed, it was recently shown that in cells, which received signals via the Fas receptor in the presence of caspase inhibitors, the phenotype of death was merely switched from apoptosis to necrosis (189). Similarly, in cells overexpressing Bcl-2, cell death induced by oxidized low density lipoproteins shifted from apoptosis to necrosis (190). It is also conceivable that apoptosis is so far down the cascade of irreversible and irreparable changes in some diseases that blocking this process may not halt the progression of the disease itself. In fact, interference with the orderly phagocytic clear-

**FIG. 2.** Coup de grace Therapeutic strategies based on modulation of apoptosis.
ance, which is part of the apoptotic program, could lead to necrotic cell lysis, which would trigger harmful inflammation. The recent report of Fas-dependent apoptosis and subsequent pulmonary fibrosis in mice (191) may perhaps be interpreted as a case of excessive apoptosis which has overwhelmed the phagocytic capacity of the tissue with ensuing "secondary" necrosis and inflammation. However, an elegant study in the fruit fly indicates that suppression of apoptosis may, in fact, constitute a viable therapeutic approach. Davidson and Steller (192) employed a Drosophila model of retinitis pigmentosa, and showed that the retinal degeneration due to mutations in either rhodopsin or a rhodopsin phosphatase gene occurred by apoptosis and could be blocked by caspase inhibitors with subsequent restoration of visual function in otherwise blind flies. Further proof of the principle that the suppression of apoptosis can result in improved cellular function was provided by J. Ilka et al. (193), who demonstrated that the increased bone mass caused by administration of parathyroid hormone (PTH) is due, at least in part, to the salvage of osteoblasts by prevention of apoptosis. To conclude, we are confident that in terms of understanding the pathogenic mechanisms underlying human maladies, we are not at the fin de siècle. We are, instead, at a new beginning. Moreover, we anticipate that apoptosis-based strategies will be rapidly incorporated into our future therapeutic arsenal.

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