Review

Mechanisms of Glucocorticoid-mediated Apoptosis in Hematological Malignancies

Stephanie Greenstein,[1] Kulsoom Ghias, Nancy L. Krett, and Steven T. Rosen

Division of Hematology/Oncology, Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois 60611

Abstract

Although glucocorticoids (GCs) have been used for their immunosuppressive, anti-inflammatory, and cytotoxic effects for many years, their precise mechanism of action has not been fully elucidated. Evidence indicates that GCs induce apoptosis in hematological cells, thus supporting their use as chemotherapeutic agents for leukemias, lymphomas, and myeloma. Although much research has been focused on investigating the mechanism of action responsible for GC-mediated cell death, the signaling pathways remain unclear. Two schools of thought have developed to account for GC-induced apoptosis. One supports the hypothesis that apoptosis is achieved via activation of death-inducing genes. The second theory states that GCs induce apoptosis via repression of transcription factor activity, thereby inhibiting the transcription of growth/survival genes. This review will attempt to clarify the complex signaling pathway responsible for mediating GC-induced apoptosis of hematological cells and to summarize the most current research in this field.

Introduction

GCs[2] are steroid hormones produced by the adrenal glands after cytokine stimulation of the hypothalamus-pituitary-adrenal axis. All natural steroid hormones share a common chemical structure and have additional chemical groups bound to the steroid nucleus that confer specificity to their actions. Dex, a synthetic steroidal GC, is a multiring structure with an added fluorine atom (Fig. 1*A*). Fluorine increases drug potency by slowing metabolism and also increases the affinity of Dex for its receptor (1). GCs are involved in the regulation of a variety of biological processes, including immune responses, metabolism, cell growth and proliferation, development, and reproduction. GCs have significant antiproliferative effects, which have led to their use for immunosuppression, treatment of inflammation, and induction of cytotoxicity. GCs induce apoptosis and have become key elements in the treatment of many hematological malignancies including leukemias, lymphomas, and MM (2–4). Despite their prevalent clinical use, the mechanisms by which the GCs induce programmed cell death have not been clearly defined, and thus GC effects require further investigation.

Glucocorticoid Receptor Domain Structure and Function

The GR is a phosphoprotein of M_r 85,000–97,000 that is present at low concentrations (10^{-4} M to 10^{-5} M of total cellular protein) in most mammalian cells (5). The GR is a member of the steroid/thyroid hormone receptor superfamily that also includes receptors for the sex steroids, thyroid hormone, vitamin D, mineralocorticoids, and retinoids (3). It is a ubiquitously expressed, ligand-dependent transcription factor that affects growth, development, metabolic functions, and stress responses. Although the GR is primarily expressed as a nuclear receptor, a cell membrane-associated receptor has been described recently (6). However, little is known about its function or its mechanism of action. The nuclear GR regulates the activities of a large number of genes by binding as a homodimer to specific DNA sequences, known as GREs, and either activating or repressing gene transcription. Similar to other members of the steroid hormone receptor family, the 777 amino acid GR is composed of a series of discrete functional domains that mediate transcription, ligand binding, and DNA binding (Fig. 1*B*). Within the domains are shorter structural motifs involved in nuclear localization, dimerization, and protein interaction (7).

The NH_2 terminus of the GR, which is poorly conserved in both length and amino acid sequence, is known by a variety of names: hypervariable region, modulatory domain, or constitutive activation domain (3, 8, 9). This region is required for the regulation of transcription (10) and has been proposed to be involved in the discrimination between different steroid target genes through protein-protein interactions with specific transcription factors (11–13). The DBD is a highly conserved, 66 amino acid core at the center of the GR that mediates the specific binding to GREs. This region shows a very high degree of homology with other steroid receptors (12, 14–16). The DBD contains two zinc finger motifs that are common DNA binding motifs among the nuclear transcription factors. They are composed of eight cysteine residues that tetrahedrally coordinate two zinc (Zn^{2+}) ions and are required for high-affinity DNA binding (17). The COOH terminus of the GR is a very complex

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[1] To whom requests for reprints should be addressed, at Robert H. Lurie Comprehensive Cancer Center, 303 East Chicago Avenue, 8340 Olson Pavilion, Chicago, IL 60611. Phone: (312) 908-5919; Fax: (312) 908-1372; E-mail: s-greenstein@northwestern.edu.

[2] The abbreviations used are: GC, glucocorticoid; GR, GC nuclear receptor; GRE, GC response element; Dex, dexamethasone; MM, multiple myeloma; DBD, DNA binding domain; HBD, hormone binding domain; hsp, heat shock protein; TNF, tumor necrosis factor; SMAC, second mitochondrial activator of caspases; DIABLO, direct IAP binding protein with low pI; IAP, inhibitor of apoptosis proteins; Apaf, apoptosis activating factor; IGF, insulin-like growth factor; AP-1, activator protein-1; NF-κB, nuclear factor-κB; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; RAFTK, related adhesion focal tyrosine kinase; STAT3, signal transducers and activation of transcription 3.

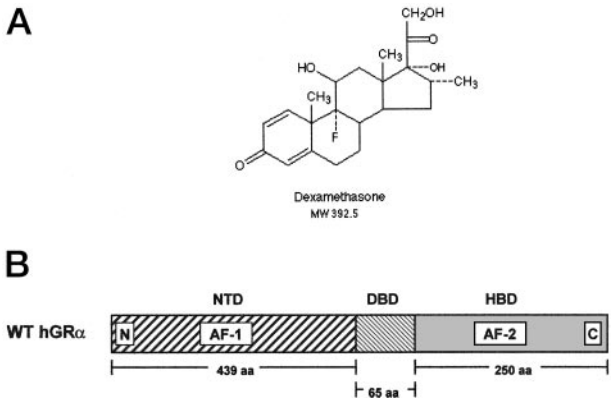

Fig. 1 *A*, steroid structure. Dex is the most common synthetic glucocorticoid. It is a four-ringed structure with a fluorine at the C9 position. *B*, schematic of the domain organization of the wild-type human GR. The GR consists of three distinct structural and functional domains: an NH_2-terminal domain (*NTD*), a *DBD*, and a *HBD*. The regulatory NTD spans 439 amino acids and contains a transcriptional activation function (AF-1) that is ligand independent. The central DBD spans 65 amino acids and contains two zinc finger motifs that are common DNA binding motifs among the nuclear transcription factors. The COOH-terminal HBD is 250 amino acids in length and contains a second transcription activation domain (AF-2) that is ligand dependent and highly conserved. *WT*, wild type; *aa*, amino acid.

structure responsible for ligand binding known as the HBD. As well as mediating hormone binding, this region contains sequences necessary for nuclear localization, receptor dimerization, and transcriptional activation (7). This domain is also involved in protein-protein interactions, such as GR binding to coactivators, corepressors, and chaperone molecules such as hsp90.

The classical effect of GCs is exerted at the gene level via the GR-GRE interaction, resulting in transcriptional induction. This occurs via two transcriptional activation sequences, AF1 and AF2. Both sequences are rich in acidic residues, which are likely required for transcriptional activation (17). The domains within the GR are also involved in nuclear localization, receptor dimerization, and hsp90 interaction. Two nuclear localization signals, NL1 and NL2, have been identified. NL1 is a 28 amino acid sequence closely related to the DBD (18). NL2 is a poorly described sequence present within the HBD. The putative dimerization signal is found in the COOH terminus of the GR; however, requirement for this signal for transcriptional activity is controversial (19). The final function of the GR is its involvement in protein-protein interactions, such as binding to hsp90, coactivators, and corepressors. A binding site for hsp90 is present within the last 100 amino acids of the HBD (20).

Mechanism of Action of Glucocorticoids

In its unliganded resting state the intracellular GR protein exists in the cytoplasm as a heterooligomeric complex containing one steroid binding protein and a multisubunit nonsteroid binding complex in a conformation able to bind lipophilic hormone ligand (21). Ligand binding induces a conformational change in the GR, which releases the receptor from its chaperones, containing hsp90 and other molecules. The release of these inhibitory molecules allows the GR to translocate to the nucleus, where it then binds to a GRE and acts as a modulator of transcription. Once bound to the appropriate response element, the GR regulates transcription of its target genes. Genes that are positively regulated by GR are characterized by responsive GR elements in their promoter regions. The GR can also inhibit gene transcription by acting as a repressor either through interaction with a negative GRE (22) or via DNA-independent interactions with other transcription factors. Numerous studies have demonstrated that intact receptor is required for apoptosis, because hematological cell lines with absent or mutant receptors evade the cytotoxic effects of GCs (3, 5, 23, 24). Thus, it is likely that lesions interfering with receptor function are a mechanism for the development of steroid resistance.

Apoptosis

For GCs to have therapeutic value as chemotherapeutic agents, they must kill susceptible cells in a manner that does not elicit a systemic inflammatory response. This mode of programmed cell death is known as apoptosis. Apoptosis is an internally encoded suicide program shared by the differentiated cells of multicellular organisms (25, 26). Apoptosis regulates the elimination of cells that are no longer needed, have developed improperly, or have sustained genetic damage (25, 26). Although diverse signals induce apoptosis, a number of conserved genes regulate a final common death pathway (26). The key is that apoptosis occurs without eliciting an inflammatory response, because cells with intact membranes are removed by phagocytosis without leaking their cytotoxic cellular contents (27).

Morphological and Biochemical Features of Apoptosis

In the majority of cells, a multitude of changes occur during the apoptotic cascade of events. These morphological and biochemical events are seen as hallmarks of apoptosis and suggest the presence of an underlying conserved cell death pathway (28, 29). The general morphological changes of apoptosis include cytoskeletal disruption, cell shrinkage, and membrane blebbing (Fig. 2; Refs. 25, 26). Apoptosis is also characterized by condensation and fragmentation of nuclear chromatin, compaction of cytoplasmic organelles, dilation of the endoplasmic reticulum, and a decrease in cell volume (28). These morphological modifications distinguish apoptotic cells from cells undergoing pathological, necrotic cell death.

There are a number of biochemical events that serve as markers of apoptosis in the majority of cells. Loss of mitochondrial function is a common event that occurs in response to extracellular cues and internal insults such as DNA damage (30). During the apoptotic process, the mitochondrial inner transmembrane potential is frequently disrupted. The change in transmembrane potential leads to a physical disruption of the outer mitochondrial membrane (30, 31). This process contributes to apoptosis in many cells by allowing the release of cytochrome *c* (32, 33) and SMAC/DIABLO (Ref. 34; Fig. 2). Thus, loss of mitochondrial membrane potential ($\Delta\Psi m$) propagates downstream apoptotic signaling. Externalization of the phospholipid phosphatidylserine is another common biochemi-

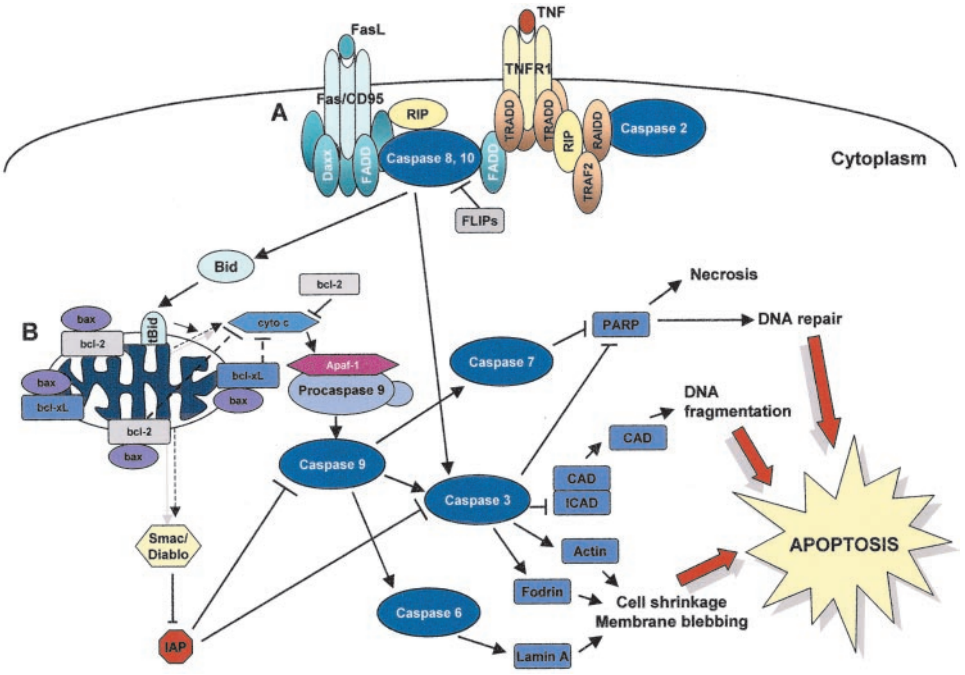

Fig. 2 Apoptotic signaling. *A,* death receptor pathway. Apoptosis is induced by the activation of death receptors, such as Fas and TNF receptor. Ligand binding promotes death receptor oligomerization and initiates the caspase cascade via specialized adaptor proteins. Fas ligand (*FasL*) binds Fas and induces receptor trimerization and the recruitment of procaspase-8 via the adaptor protein FADD. Upon autocatalysis and activation, caspase-8 stimulates apoptosis through two parallel cascades; it directly cleaves and activates caspase-3, and it cleaves the proapoptotic Bcl-2 family member, Bid. *B,* mitochondrial pathway. Members of the Bcl-2 family of proteins regulate apoptosis by altering mitochondrial membrane permeability and cytochrome c release. Activation of the apoptotic cascade is inhibited by the action of Bcl-2 and Bcl-xL, which inhibit the release of cytochrome c. The proapoptotic Bcl-2 protein Bax translocates to the mitochondria in response to death stimuli, including growth factor withdrawal, and promotes the release of cytochrome c. Truncated Bid (tBid) also translocates to the mitochondria upon activation by caspase-8, where it stimulates cytochrome *c* release and the activation of caspase-9. The subsequent activation of the effector caspases, caspase-3, caspase-6, and caspase-7, leads to the cleavage of cytoplasmic targets, causing cell shrinkage, membrane blebbing, DNA fragmentation, and eventually, apoptosis. The proapoptotic protein SMAC/DIABLO is also released from the mitochondria into the cytoplasm, where it binds IAPs and prevents the inhibitory action of this family of proteins on caspase-9 and caspase-3.

cal marker of apoptosis. The expression of phosphatidylserine on the outer leaflet of the cell membrane is a relatively early marker of apoptosis that targets dying cells for removal by phagocytic cells (35).

Caspases

Most, if not all, of the morphological changes of apoptosis are caused by a set of cysteine proteases termed caspases, which are activated specifically in apoptotic cells. These death proteases, part of a large protein family (28, 36–38), are highly conserved through evolution and can be found in organisms ranging from nematodes and insects to humans (28, 38). All known caspases possess an active-site cysteine and cleave substrates at aspartic acid residues (28, 38). Substrate specificity is conferred by the four residues NH_2-terminal to the cleavage site (28, 39). Caspases have been divided into subfamilies based on their substrate preference, sequence homology, and structural similarities.

Caspases are believed to be the central executioners of cell death, because they are they are clearly involved in the propagation of the apoptotic signal (Refs. 25, 26; Fig. 2). Once activated, they cleave critical cellular substrates and cause the hallmark changes of apoptosis. Several important caspase substrates have been identified recently. Caspase-activated DNase is the nuclease responsible for the fragmentation of DNA into a nucleosomal ladder (40). Activation occurs by caspase 3-mediated cleavage of the inhibitory subunit, which results in the release and activation of the catalytic subunit (28, 39). Other examples of caspase involvement in the morphological changes of apoptosis are the cleavage of cytoskeletal proteins such as fodrin and gelsolin (39), which results in cellular shape changes, and the proteolysis of nuclear lamins, which is required for nuclear shrinking and budding (28). Thus, the activity of the caspase enzymes is directly related to the evolution of apoptosis.

Caspase Activation

The focus of this review is the mechanism of GC-mediated apoptosis in hematological malignancies. Thus, it is important to discuss the role that caspases play in GC-induced apoptotic signaling in normal and malignant hematological cells. The participation of effector caspases, caspase-3, caspase-6, and caspase-7, in GC-mediated apoptosis has been well defined (41–44), and several reports have implicated caspase-3 as the key effector caspase. Robertson *et al.* (42) used the antiapoptotic baculovirus P35 to show that caspase-3 was activated during GC-mediated apoptosis in human pre-B leukemic cells. Using a

mouse lymphoma cell system, McColl *et al.* (41) published findings that implicated caspase-3 activation in the induction of Dex-induced apoptosis. This study, however, did not rule out the possible involvement of other caspases. Evidence suggests that there is overlap and/or redundancy between members of the different caspase families. Indeed, caspase-3 knock-out experiments have provided proof that caspase-3 is not necessarily required to mediate Dex-induced cell death (45). In addition, Miyashita *et al.* (46) have suggested that caspase-6, and not caspase-3, is cleaved and functionally active during GC-mediated apoptosis in human pre-B leukemic cells, and that caspase 3-like proteases are involved in DNA fragmentation but not Dex-induced cell death.

The identity of the inducer caspase that activates the effector caspases is similarly debatable (44). The caspase cascade can be activated via two major pathways (Fig. 2). The first, known as the death receptor pathway, involves ligand binding and receptor activation of a family of cell surface molecules called death receptors (Fig. 2*A*). Specific molecules such as Fas ligand and TNF bind death receptors (Fas and TNF receptor) and initiate a signaling cascade that results in the activation of the upstream inducer caspase-8. The second pathway, the mitochondrial-mediated pathway, is highly complex and not as well understood (Fig. 2*B*). It uses the mitochondria as a molecular relay station that transduces an incoming death signal into stimulus for activation of another inducer caspase, caspase-9 (44, 47). Substantial evidence suggests that GC-mediated cell death uses the mitochondrial pathway to initiate caspase activation. Using cells stably transfected with cowpox virus protein crmA, a caspase-8-specific inhibitor, Geley *et al.* (48) have shown that Fas-induced apoptosis and cleavage of poly(ADP-ribose) polymerase, a downstream caspase target, were blocked in CCRF-CEM human acute T-cell leukemia cells. In contrast, crmA expression did not affect GC-induced poly(ADP-ribose) polymerase cleavage or apoptosis (48). In human pre-B leukemic cells, antibodies did not detect caspase-8 cleavage and activation after GC treatment, although caspase-8 was known to be present in the cells (46). Studies using caspase-8 null mice all suggest that the membrane death receptors are not involved in GC-mediated apoptosis, and that caspase-9 is likely the initiator caspase functioning in this pathway.

But, the question remains, are the caspases direct targets of GC regulation? The fact that caspase inhibitors only delay GC-mediated cell death and do not affect long-term survival suggests that caspases are neither the sole nor the direct targets of GC action (44).

Bcl-2 Family Proteins

The M_r 25,000–26,000 Bcl-2 protein is encoded by the *B-cell lymphoma/leukemia-2* gene and is a member of another vast family of well-conserved regulatory proteins involved in apoptosis. It shares structural and functional homology with the nematode *Caenorhabditis elegans ced-9* gene and is a prosurvival component of the mitochondrial death program. Bcl-2 was first discovered as the product of an activating chromosomal translocation in follicular lymphoma. This t(14;18) translocation juxtaposes the *bcl-2* gene, normally located at 18q21, to the enhancer elements in the immunoglobulin heavy chain locus at 14q23 and results in the overexpression of the *bcl-2* gene product (49).

Bcl-2 not only plays an undeniable role in hematopoietic cell development but also in the emergence, progression, and chemosensitivity of hematological malignancies (50, 51). Bcl-2 overexpression has been implicated in the rescue of cells targeted for death by numerous stimuli, including GCs (49). The prosurvival role of Bcl-2 against GC-mediated apoptosis has been demonstrated in several systems using *bcl-2* gene transfection and differential expression studies (50, 52–57).

Bcl-2 is a unique oncogene in that it promotes cell survival rather than cellular differentiation and proliferation (49). A potential mechanism by which Bcl-2 and related proteins exert their prosurvival function is the inhibition of caspase-activating proteins such as Apaf-1 (58). There is also evidence that Bcl-2 is involved in the regulation of cytochrome *c* release (59) and intracellular Ca^{2+} partitioning. Bcl-2 can prevent Bax and Ca^{2+} from triggering the release of cytochrome *c* (and ATP) from the mitochondria, which is involved in facilitating the recruitment and activation of caspase-9 by Apaf-1, thereby inhibiting the downstream activation of the effector caspases (caspase-3, caspase-6, and caspase-7; Refs. 30, 58, 60, 61). The relative expression of Bcl-2 to Bax, a proapoptotic protein, has been implicated in regulating the response of cells to apoptotic stimuli and conferring resistance to drug-induced cell death. The apoptotic sensitivity of several hematological malignant cell lines is determined by this "molecular rheostat"; Bcl-2 promotes cell survival, and Bax promotes apoptosis (62–64).

SMAC/DIABLO

Other effector and signaling molecules known to be involved in the apoptotic cascade may conceivably play a role in GC-induced cell death. SMAC is one such effector molecule that could be a potential target of GC-mediated transcriptional regulation and has therefore received attention. SMAC, also called DIABLO, inhibits the IAP. Members of the IAP family of proteins prevent aberrant apoptosis in cells that are not destined for death by interacting with and inhibiting the enzymatic activity of mature caspases. In cells that are marked for apoptosis, however, the IAPs must be functionally inactivated and their inhibitory effect relieved. For this purpose, SMAC, which is synthesized in the cytoplasm and stored in the intermembrane space of the mitochondria, is released with cytochrome *c* into the cytoplasm when the $\Delta\Psi m$ is disrupted. Although cytochrome *c* interacts with and positively regulates the activation of Apaf-1 and caspase-9, SMAC binds to XIAP, c-IAP1, c-IAP2, and survivin and suppresses their inhibitory effect on caspase-9 and caspase-3 (65, 66).

Recently, Chauhan *et al.* (34) reported that SMAC is released into the cytosol, where it activates caspase-9 without the simultaneous release of cytochrome *c* and oligomerization of Apaf-1 in Dex-treated MM cells. This followed earlier reports that implicated caspase-3 activation and excluded the involvement of cytochrome *c* in Dex-induced cell death (67, 68). In the new study, Dex treatment caused accumulation of SMAC in the cytosol and the specific activation of caspase-9. The data suggest that SMAC forces the dissociation of the XIAP/caspase-9 complex and binds the inhibitor, allowing caspase 9 to activate

the downstream effector caspase, caspase-3. This sequence of events is abrogated upon treatment with IL-6, a known inhibitor of Dex-induced apoptosis in GC-sensitive MM cells. It would appear that IL-6 confers steroid resistance in these cells by preventing the release of SMAC, thereby inhibiting the activation of the caspase cascade and subsequent cell death. This study was not only instrumental in delineating the Apaf-1/cytochrome *c*-independent mechanism of caspase- 9 activation, but it also identified novel therapeutic targets such as SMAC and XIAP for the treatment of GC-resistant hematological malignancies (34).

Mechanisms of GC-induced Apoptosis

Although GCs have long been used as anti-inflammatory agents and anticancer treatments because of their ability to induce cell cycle arrest and cell death, the precise mechanism of action has not yet been elucidated. Although it is generally believed that a specific set of signals is involved in committing cells to apoptosis, two schools of thought have arisen from the study of GC-induced cell death. The first theory is the belief that the GCs initiate the apoptotic cascade via activation of transcription of death-specific genes. The theory is that ligand-activated GRs directly bind to *cis*-acting sequences of DNA (GREs), which function as inducible enhancer elements. This induction of transcription leads to increased expression of an apoptosis-inducing gene(s) that generates an apoptotic signal and initiates the cascade. However, to date no proapoptotic genes have been identified as targets of GC-mediated transcription. Current thinking is that GC-induced genes are responsible for the myriad of side effects associated with GC treatment (3), rather than the cytotoxic and immunosuppressive actions (69). The second theory proposes that apoptosis is initiated via negative modulation of proinflammatory cytokines or so-called survival genes, which occurs via inhibition of transcription rather than transactivation. Additionally, there is some evidence that GC-induced apoptosis is merely a consequence of cell cycle arrest. All three theories will be further discussed in this review.

GC-induced Cell Cycle Arrest

Many of the genes affected by GC treatment are critical for progression of the cell cycle, especially the G_1 to S-phase transition. Cell cycle regulators modulated by steroids include cyclin D3 and c-*myc*. Part of the chemotherapeutic potential of the GCs may be attributable to their inhibitory effect on cell cycle progression. Both apoptosis and proliferation are necessary to maintain cellular and tissue homeostasis. Cell cycle regulators link these two processes, and both are affected by apoptotic stimuli. GCs are known to lead to G_1 cell cycle arrest in human leukemic T cells (70) and transformed lymphoid cell lines (71). Cell cycle arrest on its own may serve as an apoptotic signal, especially in highly proliferating cancer cells. Conversely, inhibition of cell cycle progression may be part of a sequence of events that ultimately contribute to cell death. Decreased expression of c-*myc* and cyclin D3 is essential for GC-induced cell death in proliferating cells (71, 72). Additionally, cell cycle arrest by GCs has been attributed to induction of $p21^{Waf1}$ expression in several hematological malignancies (73). Other G_1 regulators such as E2F, p53, and Rb have also been implicated in apoptosis, further connecting the cell cycle to programmed cell death. Together theses pieces of information suggest that GC-induced cell cycle arrest is mediated by repression of cell survival and proliferative factors.

Rogatsky *et al.* (72) demonstrated that distinct domains of the GR mediate cell cycle arrest in two human osteosarcoma cell lines. They also showed that receptor activation and cell cycle arrest are coupled to cell type-specific changes in the pattern of gene expression (72). These differences might reflect differences in the regulatory proteins present in the cells but also may indicate that cell cycle arrest is not the key event in GC-induced apoptosis. Most investigators have found is that although cell cycle arrest increases the sensitivity of the cells to GCs and potentiates apoptosis, it does not in itself constitute a death signal. Therefore, it is unlikely that GR-mediated apoptosis is merely a consequence of GC-induced cell cycle arrest.

GC-induced Apoptosis and Activation of Gene Expression

In an attempt to answer the controversial question of whether the GR acts through transactivation or transrepression of gene expression, efforts have been aimed at identifying candidate genes that may be regulated either directly or indirectly by GCs. Although as yet no GR-inducible proapoptotic genes have been identified, many proteins are modulated by GCs at the transcriptional and posttranscriptional level when cells undergo GC-induced apoptosis. Thus, investigators continue to search for novel target genes involved in the GR-mediated apoptotic pathway.

In work done in our laboratory and by others, cDNA microarray hybridization technology was used to look at GC-mediated changes in gene expression. We screened the well-characterized, steroid-sensitive MM cell line (MM1.S) for Dex-induced changes in gene expression. From the 9500 human genes screened, we observed that in response to Dex exposure, 10 genes were up-regulated, and 26 genes were down-regulated 2.5-fold or greater (74). Verification of gene expression by reverse transcription-PCR and assessment of biological significance on induction of apoptosis is currently underway. De Vos *et al.* (75) identified intercellular signaling genes that were overexpressed in malignant plasma cells but not in a control autologous B-lymphoblastoid cell line. Chauhan *et al.* (76) looked at Dex-induced gene expression in the MM1.S cell line as well. They demonstrated that Dex triggers early transient induction of genes involved in cell defense and repair, followed by induction of genes known to mediate apoptosis. They also compared the gene profiles of steroid-sensitive and steroid-resistant MM cells and identified genes that may confer GC resistance. Thus, it is possible that one or more of the genes identified by oligonucleotide array may be causally involved in GC-induced apoptosis. The following section will examine the transactivation theory in the GC-mediated cell death cascade.

Interaction with p53

The tumor suppressor p53 is functionally inactivated with high frequency in human cancers, either directly by gene deletion or mutation events or indirectly because of elevated Mdm-2 levels. Mdm-2 normally interacts with, and maintains, the transcription factor p53 at low levels by signaling its degradation. In

cancers, Mdm-2 is responsible for sequestering the functional p53 protein away from its transcriptional targets that include a number of genes controlling cell cycle progression. Popularly dubbed "the guardian of the genome," p53 controls the G_1 to S-phase transition and prevents cell cycle progression at the G_1 phase in response to DNA damage. p53 is also involved in apoptosis. Although the decision between growth arrest and apoptosis after p53 activation is cell type and environment specific, the net result of the two cellular responses is the same. Evidence suggests that p53-induced growth arrest induced by DNA damage leaves the cell functionally inert. It has been established that p53-mediated growth arrest involves the activation of the cyclin-dependent kinase inhibitor, p21. However, the mechanism by which p53 induces apoptosis is still unclear. Some reports suggest that p53 transcriptionally regulates critical signaling molecules including Bax, IGF-I, and IGF binding protein 3. However, in some cases of cell death, evidence also exists for the involvement of p53-mediated transrepression and nontranscriptional events (71, 77).

Given its importance in cellular regulation and disease onset and progression, it is not surprising that researchers have looked for a link between p53 activation and GC-induced apoptosis. Ideally, this link would satisfactorily imply that p53 is up-regulated by GCs and involved in the activation of the apoptotic cascade. One of the most promising premises for this investigation is that p53 is known to regulate several apoptosis genes that are involved in GC-mediated cell death. In the murine leukemia cell line M1, *bcl*-2 expression is decreased and *bax* expression increased upon activation of a temperature-sensitive mutant *p53* gene. Bcl-2 and Bax are two important transcriptional targets of p53 that have opposing effects; whereas Bcl-2 inhibits or delays cell death, Bax promotes apoptosis (78). Therefore, p53 could play an important role in regulating the delicate balance of genes (the bcl-2:bax rheostat) that dictates cell fate upon receiving an apoptotic stimulus such as GC exposure.

In contrast, there is an abundance of data that suggest that p53 is not involved in GC-mediated apoptosis. For instance, thymocytes derived from p53 knock-out mice are resistant to apoptotic events such as ionizing radiation but are not resistant to cell death induced by GCs, suggesting that p53 is not required for apoptosis in the latter instance (79, 80). Furthermore, there is evidence to suggest that far from aiding GCs in mediating cell death, p53 may actually be involved in rendering cells resistant to GC-induced apoptosis. In particular, there is a premise that cross-talk may exist between the GR and p53 pathways in the wake of a report that wild-type p53 binds specifically to the GR *in vitro*. These data suggest that the hormone-dependent transcriptional activity of the receptor is repressed by wild-type p53 and differentially repressed by p53 mutants (81), signifying that p53 activation could be implicated in a mechanism that causes cells to become insensitive to GC-induced cell death. Indeed, one study by Mori *et al*. (82) has suggested that GC-induced apoptosis is enhanced significantly in mice thymocytes lacking one or both functional *p53* alleles.

Additional evidence for negative cross-talk between the p53 and GR pathways under physiological conditions has been reported by Sengupta *et al*. (83). In HSC-2 head and neck carcinoma cells and IMR 32 NB primary neuroblastoma cells, the GR and p53 form a strong complex in the presence of Dex and inhibit each other's transactivation properties by cytoplasmic sequestration (83). Dex treatment of hepatoma HepG2 cells under normal growth conditions and human umbilical vein endothelial cells under hypoxic conditions results in decreased protein levels of p53 and GR. Dex enhances proteasome-mediated degradation of p53 and GR by stimulating the formation of a triple complex containing the GR, p53, and the E3-ubiquitin ligase, Hdm2, and thereby inhibits hypoxia-induced *p53* gene expression and apoptosis (84). It is important to note, however, that these findings have not yet been substantiated in a hematological malignancy system.

Glucocorticoid-induced Apoptosis and Repression of Gene Transcription

Although a number of candidate genes that are down-regulated in response to GCs are also involved in apoptotic signaling induced by GCs, three specific transcription factors, which are described below, appear to be the most likely targets of GC-induced repression: AP-1, NF-κB, and oncogene c-*myc*. One very interesting aspect of the GR function is cross-talk with other proteins, such as the formation of specific protein-protein complexes with transcription factors or other components of the transcriptional machinery. The most significant interference by GCs with inflammatory and immune functions is immediate and does not require protein synthesis (85), suggesting that GR directly interferes with the signaling pathways or represses transcription factor function. Specific repression of a transcription factor can occur via competition for DNA binding sites or binding to mediators of the transcription initiation complex. This process is known as squelching (5, 86). Another mechanism for repression of gene transcription is direct sequestration of a transcription factor into its inactive form. It has been demonstrated that GR inhibits NF-κB and AP-1 in this manner (21, 87–89), which is possibly the mechanism of GC-mediated apoptosis.

NF-κB

NF-κB is a heterodimeric transcription factor that activates genes coding for cytokines, cytokine receptors, chemotactic proteins, and adhesion molecules (89), all of which are involved in the process of cell survival. The NF-κB/Rel family of transcription factors is characterized by a conserved 300 amino acid sequence called the Rel homology domain (89). In its resting state, NF-κB is sequestered in the cytoplasm by its inhibitor IκBα. IκBα maintains the NF-κB protein in an inactive conformation, until it is activated by signals such as TNF-α, UV, stress, and bacterial or viral infections. The activation cascade begins when IκBα is targeted for phosphorylation and subsequent ubiquitinylation, which identifies the inhibitor for proteasome-mediated degradation. This is followed by a conformational change in IκBα and the release of NF-κB, which allows NF-κB to translocate into the nucleus, where it exerts its influence on gene transcription (Fig. 3).

There are a number of hypotheses regarding the mechanism by which GCs inhibit NF-κB activity and induce apoptosis. Investigators have demonstrated that GCs can induce the synthesis of IκBα, which causes the retention of NF-κB within

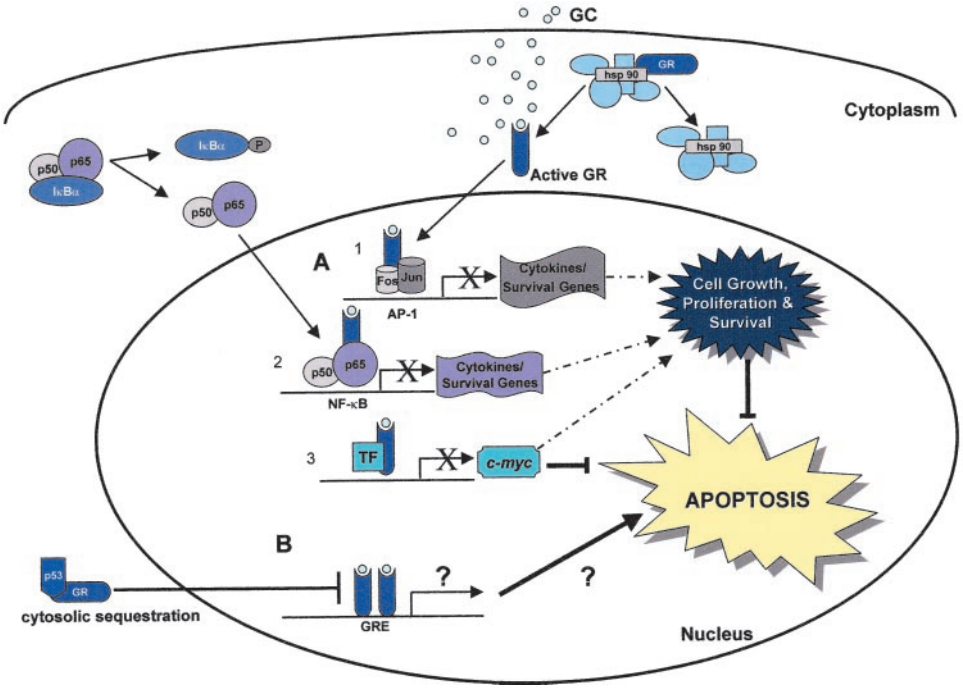

Fig. 3 Glucocorticoid-induced apoptosis via regulation of gene transcription. In the nucleus, ligated GR binds to DNA or other transcription factors (*TFs*) and modulates gene transcription via transactivation or transrepression. *A,* transrepression. *1,* AP-1 is a dimer comprised of the nuclear oncogenes, *Jun* and *Fos*. Binding of AP-1 to its response element on the DNA results in transcription of growth factors, cytokines, and survival genes. AP-1 transcriptional activity results in cell growth and proliferation. GR binds directly to AP-1 and blocks its transactivation activity, which suppresses the transcription of survival genes. Loss of survival signals leads to apoptosis. *2,* in the cytoplasm, heterodimeric NF-κB (p50, p65) is bound to its inhibitor, IκBα. Upon IκBα phosphorylation, NF-κB is released and translocated to the nucleus, where it activates transcription of cytokines, growth factors, and other survival genes. GR inhibits NF-κB transcriptional activity by: (*1*) increasing IκBα synthesis; (*2*) competing for coactivators; and (*3*) direct protein-protein interaction. Repression of NF-κB-mediated transcription of survival factors leads to apoptosis. *3,* proto-oncogene c-*myc* is involved in cell cycle regulation and proliferation and plays a causal role in cell survival. Expression of c-*myc* inhibits apoptosis and induces cell cycle arrest. GCs repress c-*myc* by an unknown mechanism. Transactivation of p53 is thought to negatively regulate GR transcriptional activity via cytoplasmic sequestration. Although the GR may induce apoptosis via gene transcription of proapoptotic proteins, the identity of these proteins is unknown.

the cytoplasm by complexing it with the newly synthesized IκBα protein (87, 90). Sequestration of NF-κB by IκBα keeps the transcription factor inactive, even under biological circumstances where it could be activated and translocated to the nucleus (87, 90). Despite evidence that GCs increase IκBα expression, it appears unlikely that this is responsible for the suppression of NF-κB activity because GC-induced inhibition is still present in the presence of the protein synthesis inhibitor cyclohexamide (91, 92). A protein-protein interaction model has also been proposed to explain GR repression of NF-κB. Several mechanistic models have been developed to account for how protein-protein interaction between GR and NF-κB results in transrepression, including mutual masking of transactivation domains and induction of posttranslational modifications. Another theory, the competition model, hypothesizes that competition for limited coactivator proteins, which mediate transcriptional activity of both nuclear receptors and transcription factors, leads to inhibition of NF-κB activity (8). However, studies have demonstrated that although coactivators are limiting for transactivation (93), GC-induced transrepression is not influenced.

Although a negative GR response element has not been discovered in any inflammatory gene promoter, interactions between GR and other transcription factors such as NF-κB does result in transcriptional interference (8, 89). This model attributes GC-induced inhibition to the direct interaction between the activated GR and NF-κB subunits within the nucleus. The p65 subunit of NF-κB and the GR have been found to associate *in vitro* (90, 94, 95) and *in vivo* (95, 96). Domain mapping and transient transfection experiments have shown that the interaction between GR and p65 involves the NH_2-terminal Rel homology domain of NF-κB and the zinc-finger motif of the DBD of GR (92, 97). The COOH-terminal domain of the p65 subunit, which contains two transactivation domains TA1 and TA2, is also needed for GR-mediated repression (91, 92). It has been speculated that activated GR disrupts essential contacts between p65 and factors of the basal transcription machinery (8, 89), a process known as tethering.

Recently, Nissen and Yamamoto (95) further clarified the mechanism of GR-mediated repression of NF-κB activity. They demonstrated that in A549 cells, the activated GR interferes with phosphorylation of the serine 2 residue of RNA polymerase II. RNA polymerase II is a component of the transcription complex, and phosphorylation at serines 2 and 5 is required for

NF-κB-induced transcription initiation (95). Thus, tethering of GR to NF-κB results in repression of NF-κB-mediated inflammatory gene transcription, which in theory could result in apoptosis. However, these findings have not yet been confirmed in hematological cell types. In conclusion, because NF-κB-dependent gene transcription is involved in cytokine regulation and apoptosis is a cytokine-regulated form of cell death, this likely represents a mechanism of action for GC-induced apoptosis.

AP-1

Another target for transrepression is transcription factor AP-1, a nucleoprotein complex that was originally discovered because it mediated the cellular responses to the potent tumor promoters, phorbol esters (98). Many signal transduction pathways converge onto AP-1 and lead to its activation. AP-1 consists of two subunits, both coded for by nuclear oncogenes, either homodimers of Jun family members or heterodimers of members of the Fos and Jun family (85). Complex formation occurs via a leucine zipper motif present in both the Fos and Jun proteins. AP-1 binds to a set of closely related DNA response elements, which results in increased transcription. AP-1 synthesis and activity is induced in response to a variety of growth factors and intracellular signals, which are mediated by protein kinases and oncogene products (99). AP-1 activity is tightly regulated via phosphorylation by intracellular signal transducers such as cyclic AMP, protein kinase A, and protein kinase C, which also play a role in mediating cell death events (100, 101).

AP-1 itself has also been shown to be involved in programmed cell death processes (99). AP-1 is activated by known tumor promoters such as phorbol esters and is down-regulated by tumor suppressors such as GCs, suggesting a pivotal role in cancer development. Because AP-1 appears to play a central role in the proliferative status of a cell (21), it is logical that it could also be involved in GC-mediated apoptosis. Jonat *et al.* (85) demonstrated that AP-1 is posttranslationally down-regulated by Dex. The question is how? Previous experiments have indicated that the inhibition of AP-1 by GCs is not attributable to blockage of or competition with AP-1 at the site of action (102). Nor is the repression attributable to coactivator competition at either the promoter or protein level (103).

Immunocoprecipitation experiments indicate a direct interaction between GR and AP-1 (85). It appears that GR and AP-1 mutually block each other's transactivating capabilities, which prevents subsequent gene transcription. Miner and Yamamoto (104) have proposed the tethering theory to explain GR-mediated repression of AP-1 transcriptional activity. Similar to the GR-NF-κB interaction, activated GR binds to AP-1 via its zinc-binding region. Rogatsky *et al.* (105) showed recently that in human U2OS osteosarcoma cells, GR associated with AP-1 through protein-protein interactions, independent of AP-1 subunit composition, and repressed AP-1-mediated transcription. Experiments performed by Helmberg *et al.* (69) demonstrated that truncated GRs lacking the NH_2-terminal activation domain were compromised in their ability to activate GRE-mediated gene transcription but were fully capable of interfering with AP-1 activity, indicating that the GC-mediated AP-1 repression does not involve the DBD of the GR. Additionally, cells expressing the mutant GR retained full sensitivity to GCs and underwent apoptosis in response to the same concentration of Dex that induces death in cells expressing the wild-type receptor (69).

A working model has been established to explain GC-induced AP-1 repression (Fig. 3). A direct protein-protein interaction between AP-1 and GR is responsible for the suppression of AP-1-mediated gene transcription of survival genes. It is hypothesized that interaction with GR leads to a conformational change of the AP-1 dimer (21), which in turn inhibits AP-1 transcriptional activity by preventing binding to the response element within the target genes. Therefore, inhibition of AP-1 activity and repression of subsequent gene transcription is critical to GC-mediated apoptosis. The question remains, what are these survival genes?

c-*myc*

It has been suggested that the proto-oncogene c-*myc* might be a survival gene involved in apoptosis. Oncogene c-*myc* is the cellular homologue of the avian cytomatosis retroviral (v-myc) transforming gene (106). It plays an important role in the control of normal cell growth and differentiation. Rearranged c-*myc* has been found in a variety of tumor types including leukemias, lymphomas, and small cell lung carcinomas, suggesting that c-*myc* plays a role in neoplastic transformation (107, 108). The mechanism of action of the Myc protein is not yet fully defined, but there is a correlation between cellular proliferation and c-*myc* gene expression. Also, c-*myc* mRNA levels are reduced in differentiating and quiescent cells relative to proliferating cells (106), suggesting a causal role for c-*myc* in cell survival.

Evidence also exists for c-*myc* participation in apoptosis (Fig. 3). Sustained expression of c-*myc* provided protection against GC-induced death in the human leukemic cell line CEM-C7, and antisense c-*myc* oligonucleotides trigger apoptosis in these cells (106). Suppression of c-*myc* was seen after treatment of T cells with a variety of steroid agents (109) and after GC treatment of a variety of normal and malignant hematological cells (55, 106, 110). Additionally, repression of c-*myc* correlated with induction of apoptosis by GCs in human leukemic cells (106). Helmberg *et al.* (69) showed that repression of c-*myc* preceded induction of GC-mediated apoptosis, suggesting that c-*myc* might be required for cell survival. Thus, c-*myc* down-regulation may be directly involved in the initiation of apoptosis in leukemic cells, and its transcription may be mediated by transcription factors like NF-κB and/or AP-1.

Other Signaling Molecules Involved in GC-induced Apoptosis

Recently, a number of other molecules in the signaling pathways of GC-induced apoptosis have been identified. Each of these signaling molecules is involved in the GC-mediated pathway, either by participating in the signal transduction or by inhibiting its progression. However, their roles relative to apoptosis have not been fully elucidated. In the next section, we will summarize what is known about these signaling molecules and their involvement in GC-induced cell death.

IL-6

IL-6 is a pleiotropic growth factor that was originally documented to induce the maturation of B cells into antibody-producing cells. It is now known to act not only on B cells but also T cells, hepatocytes, hematopoietic progenitor cells, neuronal cells, blood vessels, heart muscle, and even placenta and bone, eliciting diverse responses. In its capacity as a multifunctional cytokine, IL-6 is responsible for regulating various aspects of growth, differentiation, and proliferation in its target tissues (111). It appears that IL-6 signaling plays a regulatory role in GC-induced apoptosis in many hematological cells.

Recently, it was reported that IL-6 triggers antiapoptotic/prosurvival signals via activation of the phosphatidylinositol 3-kinase/Akt kinase pathway, and that this pathway is blocked by Dex (112). IL-6 induces Akt phosphorylation, which results in the phosphorylation and inactivation of downstream proapoptotic target molecules such as BAD, caspase-9, glycogen synthase kinase-3β, and the Forkhead family of proapoptotic transcription factors (FKHR; Ref. 112). Blocking the phosphatidylinositol 3-kinase/Akt kinase pathway leads to FKHR activation, up-regulation of $p21^{KIP1}$, and G_1 growth arrest (112). Dex has also been reported to negatively regulate *IL-6* gene expression (113, 114). At relatively high doses (10^{-6} mol/l) of the synthetic steroid, even the IL-6 receptor expression is down-regulated.

Additionally, it has been proposed that IL-6 inhibits apoptosis by interfering with the activation of stress activated protein kinase, and down-regulating the stress-induced JNK pathway and subsequent c-*jun* expression (115, 116). However, using both MM-derived cell lines and patient cells, Chauhan *et al.* (67) showed that Dex-induced apoptosis is independent of the activation of stress-activated protein kinase/JNK and p38 kinase. In contrast, Dex-induced cell death was associated with a significant decrease in the activity of both MAPK and $p70^{S6K}$ growth kinase (67) and was specifically inhibited by exogenous IL-6 expression, independent of GR activity or Dex-regulated reporter gene function (113).

Although the mechanism by which IL-6 inhibits steroid-induced apoptosis is not fully defined, new players in the IL-6 signaling pathway were identified recently. The protein-tyrosine phosphatase, SHP2, was proposed to mediate the protective effect of IL-6 against Dex-induced apoptosis in MM cells (117). The cytoplasmic domain of gp130 contains phosphotyrosine motifs for recruitment of SHP2 (118, 119), which is a positive component of growth factor and cytokine signaling pathways (120, 121), such as the MAPK pathway (122, 123). It was discovered recently that IL-6 activates SHP2 in MM cells (117), which likely results in prosurvival/antiapoptotic signaling. These studies further elucidate the signaling pathway responsible for GC-induced apoptosis and suggest that SHP2 may be a novel target for therapeutic development in hematological malignancies.

RAFTK

The signaling cascade induced by Dex has been further elucidated by identification of a new tyrosine kinase that is linked to both IL-6 and SHP2. RAFTK, also known as Pyk2, is a member of the focal adhesion kinase subfamily and is activated by stress factors such as TNF-α and UV, increases in intracellular calcium levels, activated protein kinase C, ATP (124, 125), and GCs (126). RAFTK is widely expressed in hematopoietic cells. It is rapidly phosphorylated on tyrosine residues upon activation (127, 128). RAFTK interacts with and regulates several signaling proteins, including paxillin (129), p38 MAPK (130), and JNK and Src kinases (131). Induction of apoptosis in both fibroblastic and epithelial cell lines was achieved by overexpression of wild-type RAFTK and was shown to require expression of the tyrosine kinase activity and the NH_2-terminal domain of RAFTK (132).

Recent work by Chauhan *et al.* (126) demonstrated that Dex triggered RAFTK activation and apoptosis in steroid-sensitive MM and myeloid leukemic cell lines but not in steroid-resistant MM cells. Transient overexpression of wild-type RAFTK in human MM cells induced apoptosis, whereas overexpression of the kinase inactive mutant inhibited GC-induced cell death (126), indicating that kinase activity is required for Dex-induced apoptosis. Not only does IL-6 significantly inhibit both Dex-induced activation of RAFTK and apoptosis in MM cells (126), but it also induces binding of SHP2 to RAFTK, resulting in dephosphorylation and deactivation of RAFTK (117).

Dex-induced apoptosis, but not RAFTK activation, was blocked by caspase inhibition (126), implying that RAFTK activation is upstream of caspase activation in the Dex-induced apoptotic signaling cascade. These findings support the hypothesis that RAFTK activation is an early event in GC-mediated apoptosis, and that RAFTK is critical to transduction of the apoptotic signal. These studies demonstrated that RAFTK is a substrate of SHP2, and that SHP2-mediated phosphorylation of RAFTK abrogates Dex-induced apoptosis, suggesting that RAFTK-mediated signaling is critical for GC-induced apoptosis. This information might aid in the development of therapeutic strategies to overcome GC-resistance by bypassing the blockade in RAFTK signaling.

STAT3

STAT3 is a member of the STAT family of proteins. Similar to SHP2 and RAFTK, STAT3 is linked to IL-6 via a signal transduction pathway. STAT3 is known as an acute-phase response factor, because it activates transcription of IL-6-responsive genes. Evidence suggests that constitutive activation of STAT3 occurs frequently in human tumor cells (133). Catlett-Falcone *et al.* (133) demonstrated that STAT3 is activated in bone marrow mononuclear cells from patients with MM and in the IL-6-dependent MM cell line (U266), where it appears to be essential for survival. Constitutive activation of STAT3 signaling leads to apoptotic resistance in a variety of hematological malignancies (133, 134). Additionally, inhibition of STAT3 signaling results in apoptosis in leukemic large granular lymphocytes (134). These findings strongly suggest a role for STAT3 in cell survival. Although in other nonhematological cell types STAT3 has been shown to act as a coactivator of GR by interacting with ligand-bound GR and augmenting GC-induced signaling, little is known about the STAT3-GR relationship in hematological malignancies. It can be hypothesized that GR may repress constitutive signaling through STAT3 as a way to

induce apoptosis, because STAT3 appears to be a survival signal in tumor cells. However, additional research is required before such conclusions can be made.

Calcium

There is growing evidence for the involvement of calcium (Ca^{2+}) in GC-mediated apoptosis of lymphocytes. Numerous studies have demonstrated that mobilization of Ca^{2+} is a critical step in the apoptotic pathway in thymocytes, lymphoma cells, and B lymphoblasts (133, 135–137). A recent study by Gardner *et al.* (135) demonstrated that GCs modulated Ca^{2+} homeostasis in human B lymphoblasts. In normal B cells, elevation of intracellular Ca^{2+} serves as a differentiation signal for antibody-secreting cells, IL-2 secretion, and cell proliferation. Inhibition of Ca^{2+} influx by GCs increases the susceptibility of these cells to undergo apoptosis (135). Thus, loss of Ca^{2+} from internal stores may serve as a signal to the cell to initiate the apoptotic pathway.

Investigations by Distelhorst *et al.* (136) have demonstrated that two genes that encode for Ca^{2+} channels are induced by GCs. One gene encodes for the purinergic receptor ($P2X_i$), acts as an ATP-gated Ca^{2+} channel. The second gene encodes for the phosphatidylinositol triphosphate receptor (IP_3R), which functions as an IP_3-gated Ca^{2+} channel. Both of these genes have been formally implicated in mediating apoptosis, because they have been found to be up-regulated during GC-induced cell death (136). Additionally, there is a correlation between $P2X_i$ and IP_3 receptor expression and the susceptibility of thymocytes to undergo apoptosis after GC exposure (136). These findings suggest a role of extracellular Ca^{2+} in mediating GC-induced apoptosis.

However, the signal transduction pathway initiated by cytosolic Ca^{2+} and the role it plays in GC-induced apoptosis remains unclear. Iseki *et al.* (138) demonstrated that chelation of intracellular Ca^{2+} inhibited DNA fragmentation but did not inhibit thymocyte cell death. One interpretation of these findings is that Ca^{2+} is required for endonuclease activation to mediate DNA cleavage but not necessarily for apoptosis. Another potential task for Ca^{2+} is protease activation. Ca^{2+}-dependent proteases such as calpain are activated during apoptosis of thymocytes, and calpain-inhibitors prevent GC-induced cell death (136). Other apoptotic events, such as the destruction of nuclear lamins, are also blocked by calpain inhibition. However, the caspases, which are the most prominent proteases involved in apoptosis, have not been found to be Ca^{2+} dependent. Obviously, the role of Ca^{2+} in GC-mediated apoptosis remains controversial, and more research is required for definitive answers.

Conclusion

A number of theoretical models have been proposed to explain the mechanism by which GCs induce apoptosis. This is an extremely complex signaling pathway, and it is unlikely that one single mechanism accounts for the immunosuppressive, anti-inflammatory, and cytotoxic effects mediated by the GCs. However, recent work by a variety of investigators has furthered our understanding of GC-initiated apoptosis. Despite the continued quest for evidence of GC-induced proapoptotic genes, to date no viable targets that are contributory to the apoptotic pathway have been identified. In contrast, there is strong evidence supporting the theory that GR-mediated repression of transcription of critical survival and growth factors is the mechanism of GC-induced apoptosis.

Direct interactions between GR and the transcription factors, NF-κB and AP-1, have been documented as causative events for GC-induced apoptosis. However, recent studies looking at MM cells treated with specific NF-κB inhibitors, such as PS-1145 (139) and SN-50 (140), indicate that although these agents do induce apoptosis, they do not completely abrogate cell proliferation. These results demonstrate that NF-κB is clearly important for the survival and proliferation of the cells, but that NF-κB inhibition cannot wholly account for induction of apoptosis GCs. Thus, it appears likely that GC-mediated apoptosis is the combined result of inhibition of both NF-κB and AP-1 and possible yet unidentified transcription factors.

Additionally, new signaling molecules such as RAFTK and STAT3 have been identified as participatory in the GC-apoptotic signaling cascade. Together these data indicate that GCs initiate a complex signal transduction pathway that involves multiple transcription factors and signaling molecules. Hence, continued research is needed to further define the signaling pathways initiated by GC treatment and put the signaling events into a logical chronology. Because the primary mechanism of GC resistance is loss or mutation of the GR, it is essential to design novel targeted therapeutics to bypass the need for receptor ligation. Thus, the knowledge that can be gained from additional studies will aid in the development of strategies to combat hematological malignancies.

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