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Review

Recent insights into the mechanism of glucocorticosteroid-induced apoptosis

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Abstract

Glucocorticosteroid hormones induce apoptosis in lymphocytes. Therefore, glucocorticoids are commonly used as immunosuppressive and chemotherapeutic agents. This review examines many facets of the process by which glucocorticoids induce apoptosis. This process is divided into three stages, an initiation stage that involves glucocorticoid receptor-mediated gene regulation, a decision stage that involves the counterbalancing influence of prosurvival and proapoptotic factors, and the execution stage which involves caspase and endonuclease activation. Many aspects of glucocorticoid-induced apoptosis, such as mitochondrial dysfunction and caspase activation, are important steps in virtually all forms of apoptosis. But the process glucocorticoid-induced apoptosis differs from other forms of apoptosis in terms of initiation at the transcriptional level and involvement of the multicatalytic proteasome and calcium. Moreover, the abundant opportunity for crosstalk between the glucocorticoid receptor and other signaling pathways increases the complexity of glucocorticoid-induced apoptosis and its regulation.

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Abbreviations: IL, interleukin; AP-1, activating protein-1; CTLL, cytotoxic T lymphocytic leukemia; NFAT, nuclear factor of activated T lymphocytes

Introduction

The induction of apoptosis in thymocytes by glucocorticosteroid hormones is one of the earliest recognized forms of apoptosis.[1] But progress in understanding corticosteroid-induced apoptosis has lagged behind remarkable advances in understanding other forms of apoptosis, such as that induced by the ligands of death receptors (e.g., Fas/CD95, tumor necrosis factor). In part, this is because corticosteroid-induced apoptosis is initiated by a steroid receptor-mediated change in gene expression, but specific genes that mediate cell death in response to corticosteroid treatment have not been identified.

Recent findings have shed light on events in the cell death pathway downstream from gene regulation, including the caspases responsible for the execution phase of cell death, the unexpected involvement of the multicatalytic proteasome in the death process, the suppression of prosurvival transcription factors (e.g., AP-1, c-*myc*, NF-κB), and crosstalk between T cell receptor and cytokine signaling pathways. Moreover, evidence that mitochondrial dysfunction lies downstream of caspase activation in corticosteroid-induced apoptosis challenges the generalization that mitochondrial dysfunction is the central decision point in all forms of apoptosis.

This review focuses primarily on insights gained in recent years regarding the mechanism of corticosteroid-induced apoptosis. Ultimately, the goal of investigating this form of apoptosis is to better understand the role of corticosteroid hormones in physiological regulation of the immune system and in the treatment of lymphoid leukemias and lymphomas.

To organize this review, corticosteroid-induced apoptosis is arbitrarily divided into three stages: the initiation stage, the decision stage, and the execution stage (Figure 1). The initiation stage involves corticosteroid receptor-mediated changes in gene expression, while the execution stage involves activation of caspases, or other proteases and endonucleases. The decision stage includes those events that ultimately result in a commitment to cell death. A decision to die appears to be made by counterbalancing actions of cytokines, which induce transcription factors that promote cell proliferation and survival, *versus* the actions of corticosteroids, which oppose the expression of these transcription factors. An important feature of corticosteroid-induced apoptosis is involvement of the multicatalytic proteasome, which appears to play an active role in promoting cell death by degrading factors necessary for cell survival.

The initiation stage

Glucocorticoid receptor mediated changes in gene transcription

In this review, the initiation stage is defined as the sequence of events leading up to and including direct regulation of gene transcription by the corticosteroid receptor (Figure 2). But what genes are regulated so that the lymphoid cell ultimately dies? The answer to this question has evaded investigators

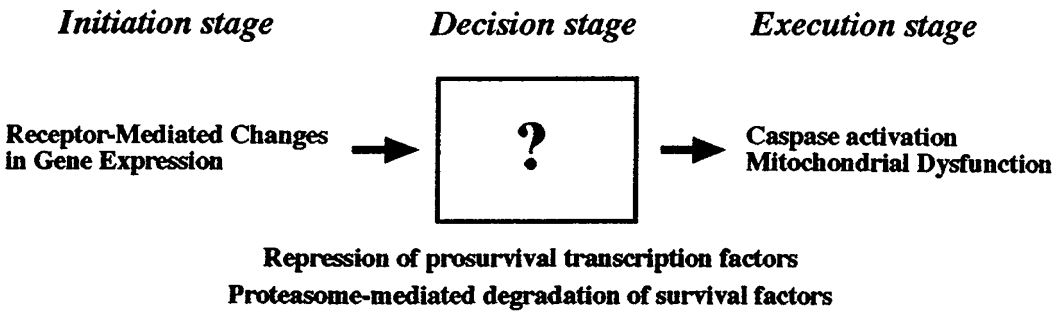

Figure 1 Stages of glucocorticoid-induced apoptosis. Between the Initiation Stage and the Execution Stage lies the Decision Stage in which counterbalancing factors determine the commitment of the cell to live or die. Recent findings indicate that repression of prosurvival transcription factors and proteasome-mediated degradation of apoptosis inhibitory factors may play a crucial role in this stage

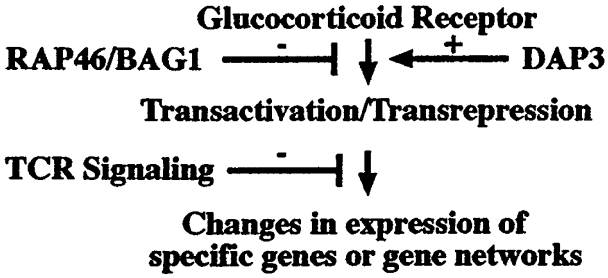

Figure 2 The Initiation Stage. There is considerable evidence that glucocorticoid-induced apoptosis is initiated by the transactivation activity of the glucocorticoid receptor, but the specific genes regulated in this process are not yet identified. RAP46/BAG1 represses the transactivation activity of the glucocorticoid receptor, and therefore inhibits glucocorticoid-induced apoptosis. Glucocorticoid-induced apoptosis is also repressed by T cell receptor (TCR) signaling, which appears to alter the transactivation activity of the glucocorticoid receptor. On the other hand, DAP3 increases glucocorticoid-induced apoptosis by enhancing the transactivation activity of the glucocorticoid receptor

up to the present time. In part, the difficulty has been knowing what to look for: the induction of a gene or set of genes that produce cell death as a direct or indirect consequence of their biological action, or the repression of genes necessary for maintenance of cell survival, or a combination of both? One specific point of confusion in understanding corticosteroid-induced apoptosis has to do with whether the transactivation or transrepression functions of the corticosteroid receptor initiate the process of cell death. Evidence in favor of each is summarized here.

Evidence for involvement of the glucocorticoid receptor transrepression activity in glucocorticoid-induced apoptosis

The concept that corticosteroid-induced apoptosis is mediated by the transrepression activity of the glucocorticoid receptor was largely based on the work of Helmberg *et al*.[2] These investigators reported that mutant glucocorticoid receptors defective in transactivation function, but fully competent at interfering with AP-1 (activating protein-1) activity, mediated apoptosis induction by corticosteroids. Since the AP-1 transcription factor regulates expression of genes involved in cell growth, differentiation and transformation,[3,4] their findings suggested that interference with transcription factors required for cell survival, such as AP-1 (activating protein-1), may contribute to apoptosis induction.[2] This interpretation is consistent with earlier findings indicating that in cytokine (IL-2)-dependent CTLL cells there is a correlation between the presence of AP-1 DNA binding activity and repression of corticosteroid-induced apoptosis.[5] In the absence of interleukin-2, corticosteroid treatment stimulates AP-1 degradation and induces apoptosis.[5]

The repression of AP-1 transcription factor activity in corticosteroid-treated lymphocytes is accompanied by repression of other prosurvival transcription factors, including the nuclear factor of activated T lymphocytes (NFAT).[6] NFAT is composed of two components, NFATp and AP-1; the dexamethasone-induced decrease in NFAT activity is due to a decrease in AP-1 activity.[6] Also, Thompson and coworkers[7] have provided substantial evidence that down-regulation of c-Myc accompanies the induction of apoptosis by corticosteroids, and that c-Myc overexpression inhibits corticosteroid-induced apoptosis.

Evidence for involvement of the glucocorticoid receptor transactivation activity in glucocorticoid-induced apoptosis

Recent evidence from several laboratories indicates that the transactivation activity of the glucocorticoid receptor is required to mediate corticosteroid-induced apoptosis. Chapman *et al*[8] found that chimeric receptors containing the potent VP16 and E1A viral transactivation domains in place of the corticosteroid receptor amino terminus enhanced the sensitivity of lymphoid cells to corticosteroid-induced apoptosis, supporting a role for transactivation in apoptosis induction. Ramdas *et al*[9] addressed the question by comparing human leukemic T cells that express either wild type corticosteroid receptor or receptor that has a mutation that is activation deficient, but that retains the ability to repress AP-1 activity. This mutant failed to mediate corticosteroid-induced apoptosis, indicating that the transactivation function of the receptor is essential for corticosteroid-induced apoptosis. In a similar approach *in vivo*, Reichardt *et al*[10] engineered a point mutation in the corticosteroid receptor in mice that impairs corticosteroid receptor dimerization, and hence inactivates

the transactivation function of the receptor. In mice with this receptor mutation, corticosteroid-induced apoptosis of thymocytes was inhibited. Since this mutant receptor retains transrepression activity, these studies provide strong evidence that the transactivation function of the corticosteroid receptor is necessary for corticosteroid-induced apoptosis.

Additional evidence for the importance of corticosteroid receptor transactivation function was provided by experiments using RAP46 to inhibit receptor function.[11] RAP46 binds to the receptor hinge region and inhibits DNA binding and transactivation by the receptor. Overexpression of RAP46 in S49 mouse lymphoma cells inhibited corticosteroid-induced apoptosis. Conversely, corticosteroid-induced apoptosis and transactivation were enhanced after treating S49 cells with the immunosuppressive rapamycin, which down-regulates the cellular levels of BAG-1, the mouse homolog of RAP46. These findings identify RAP46 as a protein that controls corticosteroid-induced apoptosis through negative regulatory action on the transactivation property of the corticosteroid receptor. In the reverse situation, the proapoptotic death-associated protein (DAP3) associates with the corticosteroid receptor and promotes corticosteroid-induced apoptosis by stimulating ligand-induced transcriptional activation by the receptor.[12]

What genes mediate glucocorticoid-induced apoptosis?

The target genes whose transactivation or transrepression initiates the cell death process remain uncertain. Candidate genes may come from unexpected sources. For example, one potential clue has been provided by the observation that thymocytes from mice homozygous for the autosomal recessive mutation Wasted (wst/wst) have increased sensitivity to corticosteroid-induced apoptosis.[13] It has been suggested that elevated thymocyte apoptosis may be a major contributor to the lymphoid dysfunction and ultimate death in wst/wst mice. Identification of the mutant gene and its function in mice with the Wasted phenotype may possibly reveal insight into genes critical for induction of apoptosis in thymocytes.

The glucocorticoid receptor, a zinc-finger transcription factor, is under autoregulatory control and its level is increased following corticosteroid treatment in human T-leukemia cells.[14] Although corticosteroid receptor upregulation appears to be an important component of cell death induction, its mechanism of action in this regard is presently unclear. Whether or not corticosteroid receptor upregulation might contribute to repression of prosurvival transcription factors is presently unknown, although this is an intriguing possibility in view of recognized cross-talk between the corticosteroid receptor and other transcription factor complexes.[15,16]

The decision stage

Multicatalytic proteasome

Recent findings have generated considerable interest in the role of the multicatalytic proteasome in corticosteroid-induced apoptosis. The proteasome is a multicatalytic protease complex located in both the cytoplasm and nucleus that degrades proteins targeted for destruction by polyubiquitination.[17–19] Although much less is known about the role of the proteasome in apoptosis, compared to the vast body of information regarding caspases, evidence in primitive organisms has suggested a fundamental involvement of the proteasome in apoptosis. For example, in the hawkmoth, *Manduca sexta*, apoptosis of intersegmental muscles is associated with elevated ubiquitin gene expression and proteasome activity.[20–22]

Genetic evidence for involvement of the proteasome in apoptosis has been provided by investigation of a dominant mouse mutation 'fused toes', characterized by partial syndactyly of the limbs and thymic hyperplasia.[23] Both morphological abnormalities are attributed to impaired regulation of programmed cell death, due to decreased expression of a novel gene, Ft1, which encodes a protein related to ubiquitin-conjugating enzymes.

In mammalian cells, evidence that proteasome activity plays a role in apoptosis induction comes primarily from work on the induction of apoptosis in thymocytes by corticosteroids. Grimm *et al*[24] demonstrated that proteasome inhibitors block corticosteroid-induced cleavage of poly-ADP ribose polymerase (PARP), a downstream caspase target, and apoptosis in thymocytes (Figure 3). These findings raised the possibility that the proteasome may either degrade regulatory proteins that normally inhibit the apoptotic pathway, or may proteolytically activate proteins that promote cell death.

In corticosteroid-induced apoptosis of thymocytes, proteasome activity appears to be involved at a step preceding mitochondrial changes and caspase activation. Beyette *et al*[25] have shown that thymocytes are rich in proteasomes, and that a chymotryptic component of proteasome activity decreases following dexamethasone treatment. Moreover, proteasome inhibitors prevented disruption of the mitochondrial transmembrane potential and also prevented exposure of phosphatidylserine and nuclear DNA fragmentation.[26] Pharmacologic stabilization of the mitochondrial permeability transition pore, or inhibition of caspases, did not prevent the activation of proteasomes.[26,27] Proteasome activation in dexamethasone-treated thymocytes is inhibited by overexpression of Bcl-2, as well as by protein synthesis inhibitors and antioxidants.[27] Involvement of the proteasome in apoptosis induction is a distinguishing characteristic of the corticosteroid-induced death pathway *versus* the Fas-mediated cell death pathway where the proteasome appears dispensable.[27]

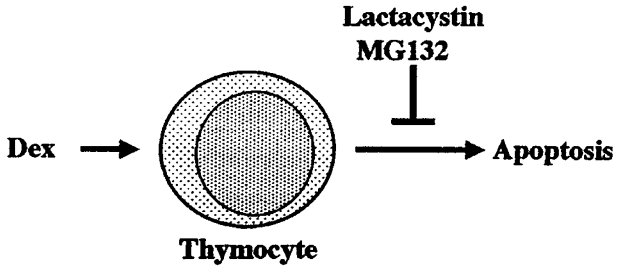

Figure 3 Proteasome inhibitors block glucocorticoid-induced apoptosis. Pretreatment of thymocytes with the proteasome inhibitors lactacystin or MG132 inhibits glucocorticoid-induced apoptosis

Although proteasome activity appears to play a proapoptotic role in corticosteroid-treated lymphocytes, proteasome targets in this context are just now being identified. One of these targets, as mentioned in the preceding section, is the transcription factor c-Fos. In a T cell lymphoma line, degradation of the transcription factor c-Fos by the proteasome was found to be a relatively early step in corticosteroid-induced apoptosis that preceded caspase-3 activation and DNA fragmentation and was inhibited by Bcl-2 overexpression.[28] A mutant form of c-Fos that evades degradation by the proteasome inhibited corticosteroid-induced apoptosis, suggesting that c-Fos degradation contributed to apoptosis induction.[28] Moreover, Ivanov *et al*[29] found that the inhibition of corticosteroid-induced apoptosis in thymocytes by the proteasome-specific inhibitor lactacystin is associated with stabilization of AP-1, NF-κB and NUR-77 against proteasome-mediated degradation.

The role of the proteasome in corticosteroid-induced apoptosis is not limited to the degradation of prosurvival transcription factors. Another concept regarding the role of the proteasome in corticosteroid-induced apoptosis comes from the work of Grassilli *et al*.[30] They found that polyamine levels decrease in thymocytes undergoing apoptosis in response to dexamethasone treatment due to accelerated degradation of ornithine decarboxylase by the proteasome. Inhibition of proteasome function preserves polyamine synthesis in association with inhibition of corticosteroid-induced apoptosis.[30]

Another target of the proteasome in corticosteroid-induced apoptosis is the cyclin dependent kinase inhibitor, p27Kip1.[31] Following treatment with dexamethasone, the level of p27Kip1 in thymocytes decreases due to proteasome-mediated degradation, a process that is inhibited by Bcl-2 overexpression and accelerated by Bax. Finally, degradation of apoptosis inhibitory proteins, c-IAP1 and XIAP, by the proteasome has been described in thymocytes undergoing apoptosis in response to dexamethasone.[32] Moreover, mutant forms of IAP that are not degraded by the proteasome had an inhibitory effect on apoptosis induction by dexamethasone, suggesting that IAP degradation promotes dexamethasone-induced apoptosis in thymocytes. The IAP's appear to bind directly to caspases and inhibit their activation.[33] Hence, the proteasome may contribute to apoptosis induction by abrogating the apoptosis inhibitory effect of IAP's.

In summary, the multicatalytic proteasome appears to play an important role in the decision of a lymphoid cell to die following corticosteroid treatment. Recognized targets of the proteasome include transcription factors that regulate genes necessary for cell proliferation (e.g., c-Fos), enzymes whose activity is essential for cell proliferation (e.g., ornithine decarboxylase), cell cycle regulatory proteins (e.g., p27Kip1), and proteins that normally repress caspases (e.g., IAP's). Bcl-2 has been reported to inhibit the increase in proteasome activity associated with corticosteroid-induced apoptosis, as well as the degradation of c-Fos and p27Kip1. Therefore, regulation of proteasome-mediated degradation of factors essential for cell survival may be a point of control for Bcl-2 in the apoptotic pathway.

Signaling pathway crosstalk

Cellular signaling pathways influence each other, producing complex integrating networks. Integration of multiple signals is important for signal transducers like the corticosteroid receptor that evoke different effects in different cells and physiologic settings.[34] Crosstalk between other signaling pathways and the corticosteroid receptor signaling pathway modulates corticosteroid-induced apoptosis, and serves as a major determinant of the cell's decision to live or die when exposed to corticosteroids. There are two ways by which signaling crosstalk might regulate apoptosis. Activation of prosurvival signaling pathways may repress corticosteroid-induced apoptosis. Alternatively, corticosteroids may repress prosurvival signaling pathways. Hence, the balance between opposing prosurvival and prodeath signaling pathways may determine the ultimate fate of cells. Several examples of this type of signaling crosstalk are summarized here.

Survival factors produced by thymic epithelial cells suppress corticosteroid-induced apoptosis.[35] More specifically, interferon alpha and interleukin-6 inhibit dexamethasone-induced apoptosis in plasma cells and myeloma cells.[36–38] Interleukins-9, -4, and -6 inhibit apoptosis in dexamethasone-treated thymocytes and thymoma cells.[39,40] Also, interleukin-15 inhibits dexamethasone-induced apoptosis in activated T and B cells[41] and insulin-like growth factors protect myeloma cells from dexamethasone-induced apoptosis.[42] Thus, a number of different cytokines counteract the induction of apoptosis by corticosteroids (Figure 4).

Interleukins may counteract corticosteroid-induced death signals by increasing expression of transcription factors that mediate expression of survival genes (Figure 5). Interleukin-2-mediated protection of T cell leukemia cells from dexamethasone-induced apoptosis correlates with induction of the DNA binding and transactivation functions of AP-1.[43] Also, interleukin-6 and interferon alpha may repress corticosteroid-induced apoptosis by activating mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways.[44]

An example of how the balance between prosurvival signals and prodeath signals works to regulate death decisions revolves around NF-κB. The NF-κB transcription factor family is required for expression of many cytokines and the immunosuppressive action of corticosteroids is

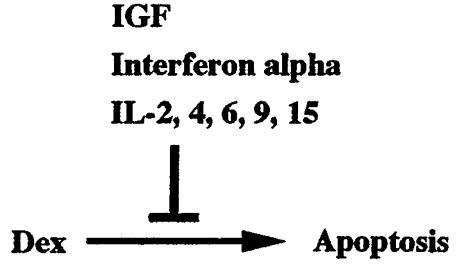

Figure 4 Cytokines counteract glucocorticoid-induced apoptosis. Insulin like growth factors (IGF), interferons, and interleukins have been shown to inhibit glucocorticoid-induced apoptosis

mediated, at least in part, through interference with NF-κB activity and, therefore, with inhibition of cytokine production. In human leukemic T cells, dexamethasone treatment induces synthesis of Iκ-Bα an inhibitor of NF-κB, which correlates with apoptosis induction.[9] In CD4+CD8+ thymocytes *in vivo*, dexamethasone treatment induces expression of Iκ-Bα and Iκ-Bβ and downregulates NF-κB DNA binding activated by intrathymic signals.[45] The downregulation of NF-κB DNA binding precedes cell death, suggesting that NF-κB may be important for the survival of immature thymocytes.[45] The negative effect of corticosteroids on NF-κB activity is counterbalanced by the cytokines, interleukins-2 and -4, that are reported to rescue lymphocytes from corticosteroid-induced apoptosis by inhibiting I-κB induction by dexamethasone.[46] In summary, this is one example of the 'Yin and Yang' of survival decisions. Corticosteroids promote cell death by inducing Iκ-Bα thereby inhibiting NF-κB activity; cytokines inhibit cell death by inhibiting the induction of I-κB by corticosteroids (Figure 6). Although interference with NF-κB activity in lymphocytes was shown to be mediated by corticosteroid induction of the inhibitor protein I-κB,[47] subsequent studies indicate that hormone-induced Iκ-B synthesis and inhibition of NF-κB activity are separable biochemical events and that the glucocorticoid receptor can directly repress NF-κB activity.[48–51]

Another example of how corticosteroid-induced apoptosis is regulated by crosstalk with other signaling pathways involves protein kinase C (PKC).[52] Protein kinase C (PKC) includes several subfamilies of enzymes including calcium-dependent protein kinase C (cPKC) and calcium-independent novel PKC (nPKC). Glucocorticoid-induced apoptosis was inhibited by non-isoform-selective PKC inhibitors but not by cPKC-specific inhibitors. Thus, nPKC isoforms appear to be involved in apoptosis induction, while activation of calcineurin and cPKC are capable of inhibiting corticosteroid-induced apoptosis. Moreover, an activator of cAMP-activated protein kinase has been reported to block dexamethasone-induced apoptosis and caspase-3 activation.[53]

T cell receptor signaling can also regulate corticosteroid-induced apoptosis. Engagement of the T cell receptor (TCR) induces apoptosis by a mechanism that involves induction of Fas ligand, which in turn interacts with its receptor and activates caspase-8.[54] Recognition that TCR signaling and corticosteroid signaling pathways are mutually antagonistic led Jamieson *et al*[55] to investigate the crosstalk pathway for inhibition of corticosteroid-induced apoptosis by TCR signaling. Their findings indicate that TCR activation of the mitogen-activated protein kinase/extracellular signal regulated kinase (MEK/ERK) cascade via Ras is necessary and sufficient to inhibit corticosteroid-mediated cell death in immortalized T cells and thymocyte cell lines, and in primary T cells. Moreover, activation of various components of the TCR pathway (Ras, MEK1) altered the transcriptional regulatory activity of the corticosteroid receptor. However, another potential explanation of how Ras represses corticosteroid-induced apoptosis may involve Ras-mediated stabilization of c-Myc protein stability. As noted above, a decrease in c-Myc protein level has been implicated as a mechanism of corticosteroid-induced apoptosis. Recently, Sears *et al*[56] reported that Ras enhances the stability of c-Myc by inhibiting proteasome-mediated degradation.

Recent findings indicate that the glucocorticosteroid receptor binds to 14-3-3, a cytoplasmic protein that interacts with a wide range of signaling molecules.[57] The corticosteroid receptor and Raf-1, a downstream effector of the signaling factor Ras, are found in a protein complex (the 'receptosome'), providing an opportunity for the corticosteroid receptor to crosstalk with the Raf-Ras signaling pathway.[58] These interactions may be involved in regulation of cell proliferation, and induction of cell death by corticosteroids, although experimental evidence to support this concept is presently lacking.

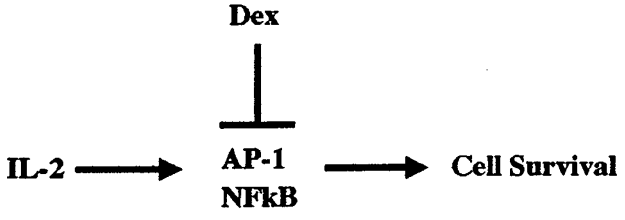

Figure 5 Cytokines increase AP-1 and NF-κB activities. Cytokines (e.g., IL-2) counteract glucocorticoid-induced apoptosis by inducing expression of transcription factors that regulate genes involved in cell proliferation

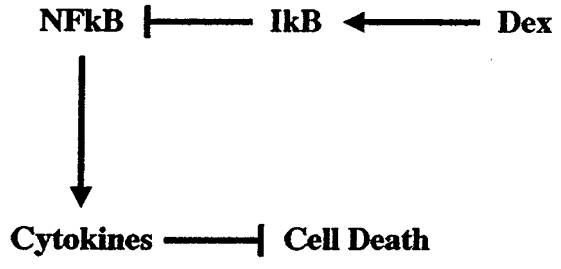

Figure 6 Glucocorticoid hormones counteract the action of cytokines. The glucocorticoid hormone dexamethasone induces expression of IκB, which inhibits the function of NF-κB, a transcription factor necessary for expression of prosurvival cytokines. Recent findings indicate that the glucocorticoid receptor may directly repress NF-κB activity independent of I-κB (see text)

Involvement of BCL-2 family members in the cell death decision

Corticosteroid-induced apoptosis is both positively and negatively regulated by members of the Bcl-2 protein family. *Bcl-2* -/- knockout mice display fulminant apoptosis of the thymus and accelerated apoptosis of thymocytes in response to dexamethasone.[59] Moreover, Bcl-2 overexpression inhibits events associated with corticosteroid-induced apoptosis, including caspase activation and mitochondrial dysfunction.[60–62]

However, the mechanism by which Bcl-2 inhibits apoptosis remains uncertain, although many hypotheses have been put forth. Currently, the most prevalent theories focus on the concept that Bcl-2 acts on the outer mitochondrial membrane to preserve mitochondrial function.[63,64] But this concept does not readily explain the antiapoptotic action of Bcl-2 in corticosteroid-treated

thymocytes, where mitochondrial dysfunction appears not to be a central mediator of cell death.

Perhaps one of the more revealing studies of the role of Bcl-2 in corticosteroid induced apoptosis was reported by Brunet *et al*.[65] These investigators employed the CEM C7A human leukemia line to investigate dexamethasone-induced apoptosis. Their findings indicate that loss of clonogenicity, which represents commitment to cell death, is separable from the appearance of caspase activation and subsequent apoptotic markers. Significantly, caspase inhibitors did not prevent commitment to cell death, but Bcl-2 did. These findings indicate that Bcl-2 works upstream of caspase activation to inhibit commitment to cell death, and that the survival function of Bcl-2 is not at the level of caspase inhibition.

A Bcl-2 family-regulated link between cell cycle and cell death has been proposed, based on recent evidence that Bax and Bcl-2 modulate Cdk2 (cyclin dependent kinase) activation during dexamethasone-induced apoptosis in thymocytes.[31] Following treatment with dexamethasone, the level of p27Kip1 in thymocytes decreases and the level of Cdk2 kinase activity increases. Cdk2 activity is crucial for induction of apoptosis in thymocytes by corticosteroids.[66] The degradation of p27Kip1 by the proteasome is regulated by the Bcl-2 family. Bcl-2 overexpression delays the degradation of p27Kip1, whereas Bax overexpression accelerates its degradation. Moreover, Cdk2 activation during apoptosis is a highly regulated process under the control of known apoptosis regulators such as Bax and Bcl-2. Bcl-2 overexpression delayed the increase in Cdk2 kinase activity associated with dexamethasone-induced apoptosis, whereas Bax overexpression had the opposite effect.

Thus, induction of thymocyte apoptosis by dexamethasone activates biochemical machinery that is shared with the normal cell cycle, i.e., Cdk2 and p27Kip1. That Bax and Bcl-2 are able to modulate the levels of p27Kip1 and Cdk2 identifies both of these factors as downstream components of a common biochemical pathway leading to apoptosis. Furthermore, these findings draw a mechanistic distinction between corticosteroid-induced apoptosis and apoptosis induction in thymocytes by Fas/CD95, as the latter does not involve changes in Cdk2 and p27Kip1 and is not inhibited by Bcl-2.[31]

Bcl-2 may also function by regulating proteasome-mediated degradation of prosurvival transcription factors. Two examples are c-Fos and NF-κB. Evidence that Bcl-2 inhibits dexamethasone-induced degradation of c-Fos by the proteasome, published by He *et al*,[28] was discussed earlier. Feinman *et al*[67] reported that dexamethasone-induced apoptosis of multiple myeloma cells is accompanied by decreased NF-κB DNA binding activity which is preserved by Bcl-2 overexpression. In this situation, Bcl-2 did not prevent I-κB induction by dexamethasone; thus, it is possible that Bcl-2 stabilizes NF-κB by inhibiting proteasome-mediated degradation.

An intriguing question concerns the involvement of proapoptotic members of the Bcl-2 family in corticosteroid-induced apoptosis. The proapoptotic family consists of members like Bax and Bak, which share significant homology with Bcl-2, and the BH3 domain only members which have only the BH3 domain in common with Bcl-2.[63] The latter family includes Bid, Bad, and Bim. A pattern of apoptosis signaling is emerging in which proapoptotic members of the Bcl-2 family transmit death signals from upstream initiator pathways to downstream effector pathways. The proapoptotic proteins are held in check by different mechanisms in the healthy cell, and then released to induce apoptosis in response to apoptotic signals.

For example, in response to engagement of death receptors by their ligands (e.g., Fas, tumor necrosis factor), Bid undergoes caspase-8-mediated cleavage which exposes its BH3 domain in the form of an active fragment that triggers mitochondrial dysfunction and cytochrome *c* release.[68] A second example is Bax, which is located in the cytoplasm and translocated to mitochondria in response to apoptotic signals (e.g., growth factor withdrawal).[63] A third example is Bad, which in the healthy cells exists as an inactive, phosphorylated form, in association with cytoplasmic 14-3-3. Apoptotic signals that trigger calcium release from intracellular stores (e.g., thapsigargin) activate Bad by inducing its dephosphorylation via the calcium-dependent protein phosphatase, calcineurin.[69] Bad dephosphorylation and activation are also induced by growth factor withdrawal. Activated Bad translocates to mitochondria, inducing mitochondrial dysfunction and cytochrome *c* release.

Although in each of these examples activation of a proapoptotic Bcl-2 family member triggers a mitochondria-dependent apoptotic pathway, other proapoptotic family members may trigger cell death through mitochondria/cytochrome *c*-independent mechanisms. For example, the BH3 peptide of Bak appears to activate effector caspases through a cytochrome c-independent mechanism.[70]

Based on evidence that proapoptotic Bcl-2 family members mediate apoptosis induction by a broad range of apoptotic signals, it is intriguing to speculate that corticosteroid-induced apoptosis may be mediated through activation of proapoptotic Bcl-2 family members. Based on findings in the Bid deficient knockout mouse, Bid is not necessary for induction of thymocyte apoptosis by dexamethasone.[71] Also, findings in the Bax deficient knockout mouse indicate that Bax is not required for dexamethasone-induced thymocyte apoptosis.[72] However, dexamethasone treatment shifts the subcellular location of Bax from a soluble to a membrane-bound form.[73] Thus, Bax may play a role in corticosteroid-induced apoptosis.

Recent findings have focused attention on the potential roles of Bak, which structurally resembles Bax, and two BH3 domain only family members, Bad and Bim, in corticosteroid-induced apoptosis. In human lymphoid leukemia cell lines, dexamethasone treatment, as well as staurosporine and etoposide treatment, induced a conformational change in Bak that preceded changes in mitochondrial membrane potential and was not inhibited by Z-VAD.fmk.[74] In chicken thymocytes, differential display analysis uncovered a nucleotide sequence that was induced by dexamethasone and that demonstrated limited homology to Bad.[75] Also, dexamethasone treatment induces elevated levels of Bax in thymocytes.[76] Another BH3 domain-only member, Bim, has been implicated in corticosteroid-induced apoptosis by studies in a Bim

knockout mouse showing that Bim deficiency inhibits the induction of apoptosis in thymocytes by dexamethasone.[77]

Thus, in the model in Figure 7, it is suggested that proapoptotic Bcl-2 family members, including Bim, Bak, Bax, or Bad, may trigger the caspase cascade. A critical question that remains to be solved is how one or more of these proapoptotic proteins are activated following corticosteroid treatment. In addition, it remains to be determined whether these proapoptotic proteins trigger APAF-1/caspase-9 activation through a mitochondria-dependent pathway involving cytochrome *c* release, or a mitochondria-independent pathway. As noted below, the current weight of evidence favors a mitochondria/cytochrome c-independent pathway to caspase activation in corticosteroid-induced apoptosis.

In summary, both antiapoptotic and proapoptotic members of the Bcl-2 family are involved in regulating corticosteroid-induced apoptosis. However, their site of action is yet to be determined. Although in many forms of apoptosis we have learned that Bcl-2 family members act primarily at the level of mitochondria, in the case of corticosteroid-induced apoptosis it appears that Bcl-2 family members may regulate degradation of cell cycle factors and transcription factors by the proteasome.

Changes in calcium and potassium homeostasis

Calcium has been implicated as a mediator of corticosteroid-induced thymocyte apoptosis for a number of years, yet the specific role of calcium in apoptosis has not been defined.[78,79] Basically, two types of evidence support a role of calcium in corticosteroid-induced apoptosis: first, inhibitors of calcium-activated proteases, or calpains, have been reported to inhibit dexamethasone-induced apoptosis in thymocytes;[80] second, alterations of intracellular calcium homeostasis have been detected in lymphoid cells undergoing corticosteroid-induced apoptosis.[60,78,79,81,82]

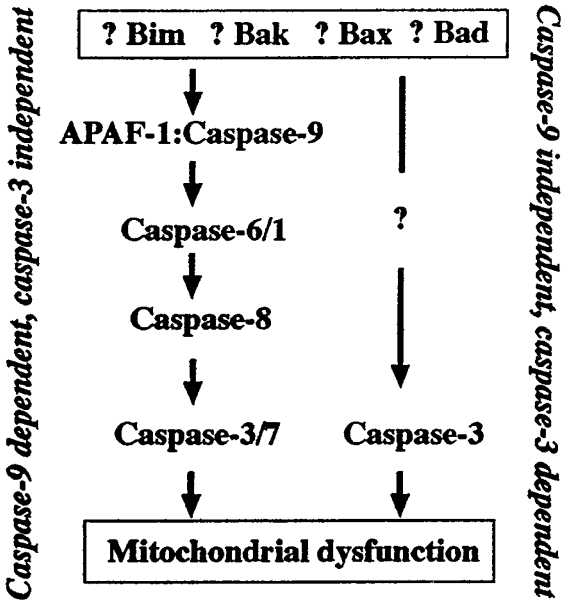

Figure 7 Proposed sequence of events in the execution phase of glucocorticoid-induced apoptosis. Proapoptotic members of the Bcl-2 family have been implicated in mediating glucocorticoid-induced apoptosis, which appears to proceed through either a caspase-9 dependent, caspase-3 independent pathway, or a caspase-9 independent, caspase-3 dependent pathway. Note that the sequence of caspase activation is different than that associated with Fas/TNF-induced apoptosis. Also, current evidence suggests that apoptosis induction in glucocorticoid-treated lymphoid cells is cytochrome *c* independent, and that mitochondrial dysfunction is a downstream event not necessary for the commitment to cell death

The major intracellular reservoir of calcium in non-muscle cells is the endoplasmic reticulum (ER). Corticosteroid treatment is associated with a decline in the calcium concentration within the ER lumen, which contributes to a decrease in total cellular calcium.[60,81,82] Moreover, recent findings have implicated the inositol trisphosphate receptor (IP3R) in corticosteroid-induced apoptosis. The IP3R is an IP3-gated calcium release channel in the ER membrane. Lymphocytes undergoing apoptosis in response to dexamethasone were found to have increased levels of IP3R expression, and antisense-mediated repression of IP3R expression was reported to inhibit dexamethasone-induced apoptosis.[83] Also, IP3R-deficient T cells are resistant to apoptosis induction by dexamethasone.[84] Therefore, these findings suggest that calcium release from the ER, via the IP3R, produces cytoplasmic calcium elevation and ER calcium pool depletion that triggers downstream effector pathways of apoptosis.

An alternative theory is that dexamethasone treatment induces expression of a plasma membrane calcium channel, the P2X receptor, resulting in an elevation of cytosolic calcium.[85] However, recent findings suggest that P2X receptor expression is not altered by dexamethasone treatment, that the P2X receptor agonist, ATP, does not induce apoptosis in thymocytes, and that P2X receptor antagonists do not block corticosteroid-induced apoptosis.[86]

The potassium ion is also implicated in corticosteroid-induced apoptosis. The potassium ion contributes to maintenance of cell volume, and volume loss is a characteristic feature of apoptosis in thymocytes.[87] Two phases of volume loss have been identified in CEM human T cell leukemia cells treated with dexamethasone: the first is a reversible phase, associated with net loss of potassium ions, while the second phase coincides with chromatin condensation.[88] Potassium efflux enhances apoptosis in thymocytes.[89] Potassium at normal intracellular levels inhibits both apoptotic DNA fragmentation and caspase-3 activation.[82] Recent findings indicate that thymocyte apoptosis is accompanied by gross perturbations of plasma membrane potential related to loss of cytosolic potassium.[90] Furthermore, an inhibitor of plasma membrane potassium channels (tetrapentylammonium) was found to be an effective inhibitor of dexamethasone-induced apoptosis. This inhibitor prevented dissipation of mitochondrial membrane potential, loss of cytosolic potassium, phosphatidylserine exposure on the cell surface, and chromatin condensation, as well as caspase and endonuclease activation.[90] These findings suggest that potassium channels contribute significantly to the regulation of some but not all pathways leading to thymocyte apoptosis.

Sphingomyelin hydrolysis and ceramide

Ceramide is produced under a variety of conditions that lead to apoptosis.[91,92] Corticosteroid-induced apoptosis is no exception, as dexamethasone treatment of normal thymocytes causes a dose-dependent increase in the concentration of endogenous ceramide.[93] The induction of ceramide appears to be secondary to induction of both acidic and neutral sphingmyelinases. Although recent evidence has pointed to phospholipid scrambling in the plasma membrane as the mechanism of sphingomyelin activation during apoptosis,[94] studies in corticosteroid-treated thymocytes suggest that sphingomyelinase activation and ceramide generation proceed by a different mechanism.[93]

In thymocytes, dexamethasone treatment rapidly induces diacylglycerol through a protein kinase C and G-protein-dependent phosphatidylinositol-specific phospholipase C (PI-PLC). This event appears necessary for acid sphingomyelinase activation.[93] The increase in ceramide was quite rapid, being detected within 5 min of hormone addition and reaching a maximum level at 15 min after dexamethasone treatment. Moreover, monesin, an endolysosomotropic agent that alkalinizes lysosomes inhibited the induction of sphingomyelinase activity, ceramide generation and apoptosis in dexamethasone treated thymocytes.[93]

These metabolic changes are inhibited by the glucocorticoid receptor antagonist RU486 and are therefore dependent upon the glucocorticoid receptor. In contrast to the recent concept that ceramide generation is secondary to caspase activation,[92] in dexamethasone-treated thymocytes caspase-3 activation was blocked by inhibitors of both PI-PLC and acid sphingomyelinase.[93] These findings argue that ceramide generation may occur upstream of caspase activation in dexamethasone-treated thymocytes.

A provocative observation was that cycloheximide and actinomycin D inhibited dexamethasone-induced apoptosis, but not dexamethasone-induced diacylglycerol and ceramide generation, and induction of caspase-3 activity.[93] Based on these findings, the authors suggest that transcription and protein synthesis are required for dexamethasone-induced apoptosis and these events are downstream in the pathway after diacylglycerol and ceramide generation and caspase activation.

Could it be that there is more than one pathway to apoptosis in corticosteroid treated lymphocytes, and that the pathway involving ceramide-mediated caspase-3 activation is one of these pathways? This concept is at least consistent with evidence, summarized below, for a caspase-3-dependent cell death pathway and a caspase-3-independent pathway in corticosteroid-treated lymphocytes.

Execution stage

Caspases

Apoptosis is mediated by caspases, a family of proteases that cleave substrates at aspartate residues. Caspases are grouped into the initiator caspases and effector caspases. For example, in Fas-induced apoptosis the initiator caspase, caspase-8, is activated by recruitment to a complex composed of the cytoplasmic portion of the Fas receptor in conjunction with adapter molecules that contain death domains. Caspase-8 activates effector caspases (e.g., caspase-3) by one of two routes: a mitochondria-dependent pathway and a mitochondria-independent pathway.[33] In the former, caspase-8-mediated cleavage of Bid, a proapoptotic member of the Bcl-2 family, converts Bid to an active form that triggers cytochrome *c* release from mitochondria. Cytochrome *c* binds APAF1, thereby activating another initiator caspase, caspase-9, which in turn activates effector caspases (e.g., caspase-3). In the mitochondria-independent pathway, caspase-8 is initially activated by the same mechanism, and then directly cleaves and activates caspase-3, bypassing mitochondria and cytochrome *c* release.

These well-established apoptotic pathways provide a conceptual model against which mechanistic features of corticosteroid-induced apoptosis can be compared. A recent mutational analysis of corticosteroid-resistant clones of the WEHI7.2 mouse lymphoma line provided genetic evidence for shared components in the dexamethasone- and Fas-mediated apoptotic pathways.[95] At a biochemical level, certain elements of the Fas pathways appear to function in corticosteroid-induced apoptosis, although a number of gaps in our knowledge blur the picture considerably.

Perhaps the most certain conclusions regarding the role of specific caspases in corticosteroid-induced apoptosis are provided by knockout mouse experiments. These experiments indicate that both APAF1 and caspase-9 are essential for corticosteroid-induced apoptosis. Thymocytes from knockout mice deficient in either *APAF1*[96] or caspase-9[97,98] are resistant to corticosteroid-induced apoptosis. On the other hand, caspase-1 deficiency[99] and caspase-3 deficiency[100] do not prevent corticosteroid-induced apoptosis. This indicates that caspase-3 is not required for corticosteroid-induced apoptosis.

Although knockout mouse studies define critical elements of corticosteroid-induced apoptotic pathway, they also reveal the complexity of the pathway. For example, in caspase-9 -/- thymocytes treated with dexamethasone, procaspases-2, -3, -7 and -8 underwent processing to a limited extent, and cleavage of PARP (poly-ADP-ribose polymerase) and Rb (the retinoblastoma protein) was observed.[97] Thus, there appear to be two alternative pathways to corticosteroid-induced apoptosis. One pathway, which appears to predominate in thymocytes, is caspase-9 dependent and caspase-3 independent, while the other is caspase-9 independent and caspase-3 dependent.

Also, knockout studies have revealed an important difference between Fas-induced apoptosis and corticosteroid-induced apoptosis. Proteolytic activation of procaspase-8 was markedly reduced in *Apaf-1* -/- thymocytes treated with dexamethasone, whereas procaspase-8 processing in response to Fas was not diminished. These findings suggest that, in the case of corticosteroid-treated thymocytes, procaspase-8 activation is downstream of Apaf-1-mediated caspase-9 activation.

Studies using caspase inhibitors complement the findings in knockout mice. Corticosteroid-induced apoptosis in both thymocytes and lymphoma cells is inhibited by the

broad spectrum caspase inhibitor, Z-VAD.fmk.[61,101–104] Also, the baculovirus p35 protein, which inhibits caspase activity, blocks corticosteroid-induced apoptosis.[105]

Although there is good agreement regarding the inhibition of corticosteroid-induced apoptosis by broad spectrum caspase inhibitors, there is disagreement regarding the effect of the more specific caspase-3 inhibitor, Z-DEVD.fmk. One report has suggested that corticosteroid-induced apoptosis can be inhibited by the caspase-3 inhibitor, Z-DEVD.fmk,[61] whereas other reports have failed to detect inhibition of corticosteroid-induced apoptosis by Z-DEVD.fmk.[104,106] Although such discrepancies may be due to differences in experimental methods, they could also arise if a caspase-3-dependent pathway predominates in certain lymphoid subtypes, whereas a caspase-3-independent pathway predominates in other lymphoid subtypes.

Moreover, reports differ with regard to results of caspase-3 activity assays following corticosteroid treatment, with one report detecting caspase-3 activation in corticosteroid-treated cells[107] and another report indicating that caspase-6, rather than caspase-3, is activated in corticosteroid-induced apoptosis.[106] Again, these findings are consistent with the results of knockout mouse studies and suggest that there may be more than one pathway to corticosteroid-induced apoptosis, one in which caspase-3 predominates and one in which caspase-6 predominates.

The relative importance of caspase-6 over caspase-3 in corticosteroid-induced apoptosis is highlighted by a recent study by Komoriya *et al*[108] using cell-permeable fluorogenic caspase substrates, with the goal of ordering the sequence of events in the caspase cascade *in vivo*. The findings of this study demonstrate that the sequence of caspase activation is different in corticosteroid-induced apoptosis compared to Fas-induced apoptosis. After dexamethasone treatment there is a pattern of sequential caspase activation with time, as expected for the caspase cascade proposed from biochemical studies, with caspase-9, followed by caspase-1, caspase-6, caspase-8, and caspases-3 and -7. The order of caspase activation for anti-Fas-treated thymocytes was less clear-cut, but distinct from dexamethasone-treated thymocytes with caspase-8 initially activated and caspase-9 activated at a latter stage.

It is important to keep in perspective that caspase activation and caspase-mediated protein cleavage are components of the execution stage of programmed cell death. In the case of corticosteroid-induced apoptosis critical events preceding caspase activation determine whether the cell is to live or to die. This point is emphasized by work showing that caspase inhibitors block proteolysis of endogenous substrates and reduce nuclear condensation, but do not alter dexamethasone-induced changes in clonogenicity. Therefore, it appears that commitment to cell death precedes key biochemical and morphological markers of apoptosis by several hours, and that separate regulators govern cellular commitment to clonogenic death and the subsequent execution phase characterized as apoptosis.[65]

In summary, based on the constellation of findings from knockout mice, inhibitor studies and caspase assays, the prevailing pathway to corticosteroid induced apoptosis appears to be caspase-9-dependent and caspase-3-independent (Figure 7). This pathway involves APAF-1-mediated activation of caspase-9 (the initiator caspase), followed by activation of caspases-1 and -6 (the effector caspases). An alternative pathway that is caspase-9-independent and caspase-3-dependent may also exist, but is less well defined. A critical question that remains to be answered is how corticosteroid treatment triggers activation of the caspase cascade and which proapoptotic Bcl-2 family members (e.g., Bim, Bak, Bax, Bad) are involved in this process.

Mitochondrial dysfunction

Dexamethasone treatment induces loss of mitochondrial membrane potential in thymocytes, followed by increased superoxide generation.[109,110] Also, dexamethasone treatment induces loss of mitochondrial membrane potential in T-cell hybridoma cells.[62] However, it appears that mitochondrial dysfunction occurs downstream of caspase activation in corticosteroid-induced apoptosis.

Perhaps the most convincing evidence for this conclusion comes from gene knockout studies. Caspase-9 deficiency in the *caspase-9* knockout mouse prevented loss of mitochondrial membrane potential following dexamethasone treatment.[97] Less certain are studies of the effect of the broad spectrum caspase inhibitor, Z-VAD.fmk, on changes in mitochondrial function in dexamethasone-treated cells. One study reported that Z-VAD.fmk prevented loss of mitochondrial membrane potential and production of reactive oxygen species,[106] whereas another study reported that Z-VAD.fmk did not prevent dexamethasone-induced changes in mitochondrial membrane potential.[65] Surprisingly, only one reported study has examined the role of cytochrome *c* release in corticosteroid-induced apoptosis. This study, employing dexamethasone-sensitive multiple myeloma lines, indicated that cytochrome *c* release was not detected in association with apoptosis induction by dexamethasone.[111]

In summary, the role of mitochondria in corticosteroid-induced apoptosis is uncertain, but based on the collective evidence available at the present time, mitochondrial dysfunction does not appear to be a central step in the initiation of the cell death pathway in corticosteroid-induced apoptosis. Although mitochondrial dysfunction is detected during the evolution of apoptosis in dexamethasone-treated cells, it appears to occur downstream of commitment to cell death, and may be induced by caspase activity (Figure 7). Thus, it will be important in future studies to look for a mitochondria-independent pathway to cell death in corticosteroid-treated cells.

Clinical actions of corticosteroids and corticosteroid resistance in leukemia and lymphoma

Corticosteroids are employed frequently in the treatment of both acute and chronic lymphocytic leukemia, and a variety of different lymphomas, due to their ability to induce apoptosis. Recent advances in understanding the mechanism of

corticosteroid-induced apoptosis have been applied to better understand the role of corticosteroids in lymphoid malignancy.

Perhaps the most revealing are studies of corticosteroid treatment in acute lymphoblastic leukemia (ALL). A recent randomized trial, reviewed by Gaynon and Carrel,[112] found that corticosteroids are clearly essential for optimal event free survival, and deferral of steroid treatment until the second month of therapy reduced survival. Also, the *in vitro* and *in vivo* response of leukemic cells to corticosteroids is highly predictive of outcome. At relapse, loss of *in vitro* sensitivity to corticosteroids is common and out of proportion to loss of sensitivity to other agents. Moreover, in a large group of children with newly diagnosed ALL, *in vitro* resistance to prednisone predicted a poor response to *in vivo* prednisone monotherapy and worse long term outcome.[113]

The mechanism of corticosteroid resistance in human leukemia and lymphoma cells is poorly understood. A recent report identifying a somatic mutation of the glucocorticoid receptor gene in leukemic blasts from a patient with acute lymphoblastic leukemia, and a cell line derived from the same patient, suggests that there may be value in searching for additional receptor mutations and testing their association with corticosteroid resistance.[114] In view of recent findings, described above, indicating that Ras activity can repress corticosteroid-induced apoptosis *in vitro*,[55] it is interesting to recall an earlier clinical study showing that activating N-Ras point mutations are detected in some cases of dexamethasone-resistant acute lymphoblastic leukemia.[115]

The possibility that expression levels of Bcl-2 family members may be of prognostic significance has also been tested in recent clinical trials. In cell lines, the Bax/Bcl-2 ratio correlated with sensitivity to dexamethasone.[116] In bone marrow derived blasts, the Bcl-2 and Bax levels were highly variable, although the Bax/Bcl-2 ratio appeared to be more important than the Bcl-2 level as a predictor of drug-induced apoptosis.

In a clinical trial of childhood ALL reported by Salomons *et al*,[117] blasts from patients with newly diagnosed childhood ALL were examined for levels of Bcl-2 protein family expression. Expression levels of apoptosis inducers (Bad, Bak) and inhibitors (Bcl-xL, Mcl-1) were highly variable in blasts from the 78 children with newly diagnosed ALL tested. Protein expression levels of the Bcl-2 family were not found to correlate with *in vitro* resistance to drugs, including prednisone. Moreover, neither blast reduction following one week of prednisone monotherapy, nor long term disease-free survival showed a correlation with Bcl-2 family protein expression.

A different conclusion was reached in a study of newly diagnosed and relapsed ALL reported by Haarman *et al*.[118] In this study, *in vitro* assays of drug sensitivity were performed at the time of diagnosis and at the time of relapse. At time of initial diagnosis, Bcl-2 expression was not associated with increased *in vitro* drug resistance. But at the time of relapse, increased expression of Bcl-2 correlated with increased resistance to corticosteroid-induced cell death.

In summary, basic understanding of the mechanism of corticosteroid-induced apoptosis is presently being applied to better understand and predict that action of corticosteroids in the clinical setting. A more in depth understanding of the fundamental cell death pathway, and its regulation, will no doubt lead to even greater insight into mechanisms of corticosteroid resistance in the clinical setting, and perhaps to novel modes of therapeutic intervention.

Summary and speculation

The mechanism of corticosteroid-induced apoptosis is only partially understood, and there are many questions to be answered. First, although there is considerable evidence that receptor-mediated regulation of gene transactivation is important, the identity of specific genes or gene networks involved in initiating the cell death process is unknown. At the other end of the spectrum, there is considerable knowledge about which caspases are involved in cell death execution, and the sequence of their activation. However, the mechanism that initiates the caspase cascade is unknown. Although certain proapoptotic members of the Bcl-2 family have been implicated, how they actually trigger caspase activation is uncertain. Although it is tempting to postulate that cytochrome c release from mitochondria is the triggering event, as it is in many forms of apoptosis, there is surprisingly little information about cytochrome *c* release in corticosteroid-induced apoptosis. The only report suggests that cytochrome *c* is not released from mitochondria during the course of corticosteroid-mediated apoptosis. Could it be that corticosteroids activate caspases through an APAF-1/caspase-9 dependent, but mitochondria/cytochrome c-independent mechanism?

Between the initiation stage of cell death and the execution stage of cell death lies a mystery. How do transcriptional changes mediated by the corticosteroid receptor lead the cell down a path to death? This review has summarized a number of events that occur in lymphoid cells following exposure to a corticosteroid hormone. Each event, when viewed in isolation, could provide basis for a hypothesis about what governs the cell death decision. However, upon closer examination at least one common theme emerges from the reported observations: corticosteroids appear to oppose the action of growth factors.

This concept is illustrated in Figure 8, and is described as the 'Yin and Yang' of corticosteroid-induced apoptosis. It may be the balance between the prosurvival action of growth factors and the proapoptotic action of corticosteroids that ultimately determines a cell's fate. Growth factors increase the activity of a number of transcription factors that mediate expression of genes involved in cell proliferation. Corticosteroids appear to decrease the activity of these same transcription factors. All of these transcription factors are targets of the multicatalytic proteasome. Hence, corticosteroid treatment of susceptible lymphoid cells may mimic growth factor withdrawal by targeting critical transcription factors for accelerated degradation by the multicatalytic proteasome. The role of the proteasome in corticosteroid-induced apoptosis is not limited to transcription factor targets. An enzyme involved in polyamine synthesis, ornithine decarboxylase, is also degraded by the proteasome in corticosteroid-treated cells, thus counterbalancing the induction of this enzyme by IL-3.

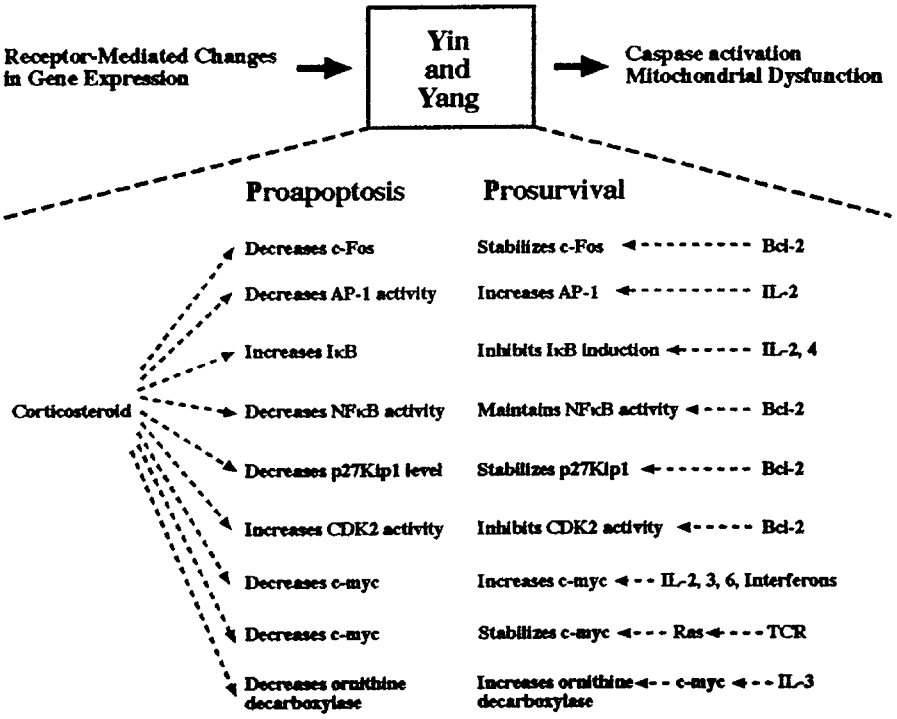

Figure 8 The 'Yin and Yang' of glucocorticoid-induced apoptosis. Cytokines and Bcl-2 favor the expression of a series of factors important for cell proliferation and cell survival, whereas glucocorticoids have the opposite effect. It appears that the cell death decision is based on the balance of these opposing actions

Although the preceding arguments suggest a mechanism by which corticosteroids may counterbalance the proliferative effects of growth factors, they do not readily explain how the decision to die is translated into caspase activation and cell death execution. One clue is that proteins that function to inhibit caspases (i.e., c-IAP1, XIAP) are degraded by the proteasome during corticosteroid-induced apoptosis. It is likely that other regulatory molecules that serve to keep either proapoptotic Bcl-2 family members or caspases in check will also be found to be targets of the proteasome during corticosteroid-induced apoptosis. Thus, although somewhat speculative at this point, it seems possible that corticosteroid-induced apoptosis is mediated by targeting a variety of prosurvival factors for degradation by the multicatalytic proteasome. Interestingly, the antiapoptotic protein Bcl-2 has been reported to stabilize several of the proteasome targets implicated in corticosteroid-induced apoptosis. Thus, it is possible that proteasome-mediated degradation of prosurvival factors may be a central, Bcl-2 regulated step in corticosteroid-induced apoptosis.

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