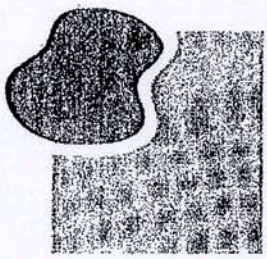

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caspases A cohort of cysteine aspartic acid-specific proteases that function either as initiators (e.g., caspase-8 and -9) or as executioners (e.g., caspase-2, -3, -6, and -7) of the apoptotic cell death program in vertebrates.

gene knockout A mutant mouse line generated by targeted disruption (inactivation) of a specific gene, generally through homologous recombination, to examine the functional significance of that gene product in cell, tissue, or organ function.

See Also the Following Articles

Apoptosis • Apoptosis, Glucocorticoid-Induced • Estrogen Receptor Biology and Lessons from Knockout Mice • Knockout of Gonadotropins and Their Receptor Genes • Placental Development

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Apoptosis, Glucocorticoid-Induced

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Glucocorticoids (GCs) play a vital role in maintaining normal metabolism, regulating various physiological processes, and overcoming several forms of stress. Because of their diverse actions, naturally occurring as well as synthetic glucocorticoids are commonly used as therapeutic agents for various disorders. In a number of physiological and therapeutic instances, induction of or protection from apoptosis is a critical aspect of GC action. Apoptosis is a form of suicidal cell death that has now been recognized as being an integral part of physiological cell turnover and helps maintain the dynamic state of cellular homeostasis. It is an energy-requiring process

typically involving altered expression or function of key cell proliferation/death genes. Excessive or defective apoptosis has been implicated in a number of diseases, provoking the development of therapeutic strategies targeting any physiological imbalance in the process. In this article, we briefly outline the mechanism of action of GCs, identify specific physiological targets of GC-evoked apoptosis, and cite examples of therapeutic applications in which GC regulation of apoptosis is relevant to its therapeutic efficacy. Finally, we present a concise review of the mechanisms of GC-evoked apoptosis, based on the extensive literature from studies on thymocytes and leukemic lymphoblasts.

I. MECHANISM OF GLUCOCORTICOID ACTION

Glucocorticoids (GCs) modulate their actions via binding to a specific intracellular GC receptor (GR), which upon ligand binding is released from the complex of cytoplasmic proteins to which it is bound in the inactive state. The GR has been shown to be essential for GC-evoked apoptosis. The classical model of GR action postulates that the activated GR is translocated to the nucleus, where it regulates transcription of a finite set of genes via GC-response elements (GREs) on target genes. Recent studies have demonstrated that this model is rather simplistic, and GR-dependent transcriptional regulation is modulated by precise interactions of the GR with "co-regulatory" proteins and involves the formation of a "transcriptosome" complex, which includes basal transcription factors (such as TBP and TFIID) and either co-activators (such as CBP and other histone acetylases) or co-repressors (such as NcoR and other histone deacetylases). Most natural GR target genes contain complex GREs, termed GC-response units, that bind multiple transcription factors either cooperatively or competitively. GR also modulates physiological processes by cross talk with other signal transduction pathways and secondary transcriptional and posttranscriptional effects.

II. GC-EVOKED APOPTOSIS IN REPRODUCTIVE TISSUES

GCs impact on normal cell turnover in several other tissues, as evidenced in experimental rodent models, in cell cultures, and in humans. In the prostate, glucocorticoids, in conjunction with androgens, stimulate glandular epithelial cell proliferation and prevent apoptosis. The relevance of GCs in the process is apparent in castration (androgen withdrawal)-induced prostate involution, which is inhibited by high doses of GCs. By a process that involves the GR, GCs prevent a castration-induced increase in the apoptotic genes TRPM-2, c-*fos*, and hsp 70. Testicular germ cells are prone to stress-induced apoptosis, mediated by increased secretion of GCs and resulting in suppression of testosterone levels. GCs modulate the cyclic pattern of epithelial cell proliferation and involution in the mammary gland in synergy with estrogens, progesterone, prolactin, and insulin. Using immortalized mammary cell culture models, GC deprivation has been shown to trigger apoptosis. Involution of postlactation mammary gland is caused by apoptotic loss of epithelial cells owing to a fall in levels of lactogenic hormones, including GCs.

III. EFFECTS OF GCs ON BONE TURNOVER

In the skeletal system, GCs influence the production and function of paracrine and autocrine factors including hormones and cytokines secreted by bone cells, the net effect being increased bone resorption and decreased bone volume and density. The primary target of GC action in the bone seem to be osteoblasts, which modulate bone formation and mineralization and eventually become osteocytes and undergo apoptosis. GCs have been shown to induce osteoblast apoptosis and repress osteocalcin, insulin-like growth factor-1, and type I collagen synthesis, all implicated in preosteoblast differentiation and proliferation. Impaired osteoblastogenesis secondarily represses osteoclastogenesis, thereby resulting in diminished bone turnover and remodeling. In addition, GCs promote osteocyte apoptosis, a primary cause of osteonecrosis (a misnomer, since the cells are actually dying via apoptosis rather than necrosis) in isolated portions of bone after administration of pharmacological doses of GCs. Indeed GC-induced bone resorption is a major cause of osteoporosis, often manifested following GC administration for inflammatory disease or disorders of immune function. Paradoxically, GCs are effective therapeutic agents for chronic inflammatory diseases of the bone, such as rheumatoid arthritis. Rheumatoid arthritis is associated with neutrophil activation, infiltration, and apoptosis at the inflamed joint. GCs prevent bone erosion by inhibiting pro-inflammatory neutrophil function including apoptosis.

IV. GC-MEDIATED REGULATION OF THYMOCYTE SELECTION AND LEUKOCYTE APOPTOSIS

Early studies demonstrated that GCs evoke thymic involution and affect the development of a normal immune system. During development, immature T-cells are subjected to a rigorous selection process in the thymus, allowing survival of cells with precisely defined properties. Double-positive, immature thymocytes that do not express functional T-cell receptors apoptose via a default pathway mediated by GCs. T-cells that do express functional T-cell receptors escape this pathway but are further subjected to negative selection if their receptors recognize self-antigen/MHC complexes with high avidity. GCs influence the TCR avidity "threshold" that distinguishes between cells that survive (low avidity) and those that die (high avidity). Indeed, in experimental models, apoptosis triggered by receptor-mediated T-cell activation is antagonized by GCs; deprivation of GC lowers the threshold of avidity beyond which negative selection ensues. GCs also affect the death of circulating leukocytes including neutrophils, eosinophils, and T- and B-lymphoid cells by mechanisms that are not clearly understood.

Because of their apoptotic actions, GCs are powerful therapeutic agents for a number of disorders, including autoimmune disorders, allergies, asthma, inflammatory diseases, and several forms of leukemia. GCs are effective components of multiagent combination therapy for ALL and CLL, where therapeutic efficacy is linked to the presence of functional GC. Eosinophil infiltration of bronchial mucosa causes many of the pathological features of asthma, including blockage of lung airways. GC-evoked apoptosis of eosinophils is instrumental in their ability to facilitate clearance of lung airways and reduce inflammation in asthma. Similarly, eosinophilia-associated allergic inflammation is also alleviated by GC-mediated eosinophil apoptosis. In autoimmune diseases, such as rheumatoid arthritis and lupus, GCs are effective therapeutic agents because of their ability to reduce the numbers of $CD4^+$ and $CD8^+$ thymocytes via apoptosis (see Fig. 1).

V. MECHANISMS OF GC-EVOKED THYMOCYTE APOPTOSIS

GC-evoked thymocyte apoptosis has been extensively studied and has facilitated a better understanding of the molecular pathway for apoptosis. Morphological

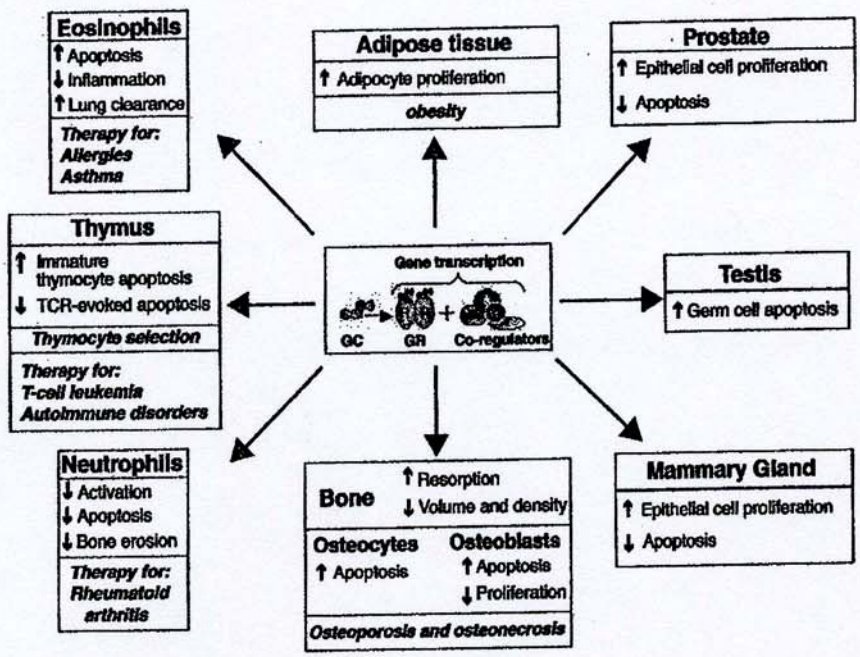

FIGURE 1 Targets of GC-evoked apoptosis and examples of therapeutic applications in which GC regulation of apoptosis occurs.

and biochemical changes associated with GC-evoked apoptosis include cell shrinkage, chromatin condensation, membrane flipping and blebbing, DNA fragmentation, and formation of apoptotic bodies. Researchers have been studying the molecular changes that precede these irreversible manifestations of apoptosis. It has become apparent that several overlapping or redundant pathways are simultaneously activated by GCs, all of which eventually culminate in protease activation, degradation of target substrates, and subsequent cell death (Fig. 2).

A. Alterations in Gene Expression

GC-mediated regulation of certain genes seems to play a vital role in evoking thymocyte/lymphoid cell apoptosis. One of the early effects is the down-regulation of expression of the proto-oncogene c-*myc*, which has been implicated as a decisive event triggering the apoptotic machinery. In GC-sensitive leukemic CEM-C7 cells constitutively expressing ectopic c-*myc*, apoptosis is significantly delayed. c-*myc* levels are not repressed in GC-resistant lymphoid cell lines or in GC-resistant thymocytes from NOD mice, providing a strong correlation between GC-evoked apoptosis and c-*myc* suppression. In murine P1798 cells, however, GC-evoked c-*myc* suppression is not sufficient to trigger an apoptotic response; a simultaneous depletion of the G1 cyclin, cyclin D3, is necessary. Indeed, GC-evoked apoptosis of leukemic cell lines is accompanied by growth arrest in the G1 phase of the cell cycle, which is thought to require regulation of cyclin–cyclin-dependent kinase (cdk) complex formation. GC-mediated lymphoid cell growth arrest/apoptosis is associated with up-regulation of the cdk inhibitor $p27^{kipI}$ and down-regulation of various cyclins, effectively blocking cdk activity.

The proto-oncogene c-*jun*, a component of the transcription factor AP-1, is up-regulated in association with GC-evoked apoptosis of lymphoid cells and may alter GR function via AP-1-mediated cross talk. AP-1 is also known to activate protein kinase C isoforms, some of which have been implicated as being pro-apoptotic. Another second messenger, cAMP, promotes GC-mediated thymocyte apoptosis by as yet unidentified mechanisms The classic path of cAMP signaling is through activation of protein kinase A, leading to modulation of gene transcription via phosphorylation of cAMP-response element-binding protein (CREB) and recruitment of CREB-binding protein (CBP) to alter gene expression of cAMP-responsive genes. Both CREB and CBP are known to interact with GR as components of the GR co-regulatory complex.

B. GC-Mediated Activation of Signaling Pathways

Induction of genes mentioned in the previous section is also accompanied by activation of multiple signal transduction pathways, including those activated via

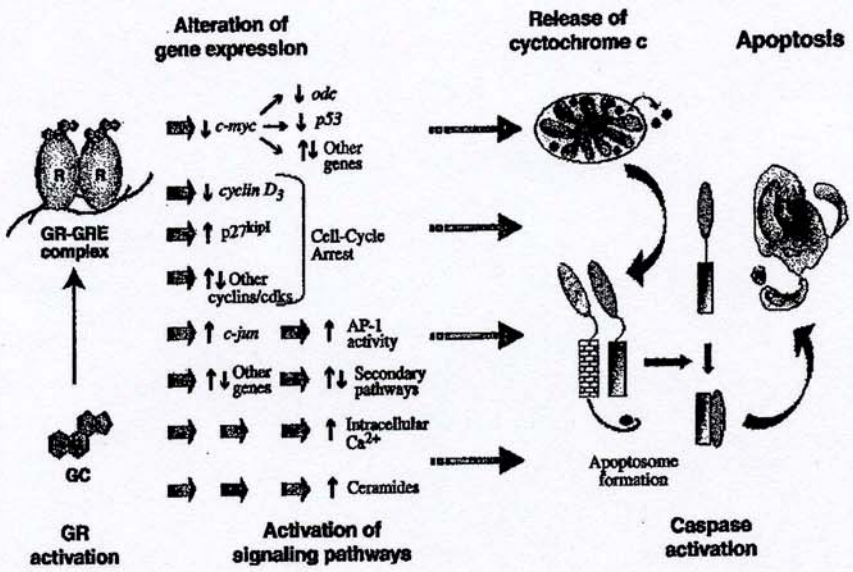

FIGURE 2 Signaling pathways that are activated by GCs, eventually leading to protease activation, degradation of target substrates, and subsequent apoptosis.

protein kinases C and A. GCs elevate cytosolic Ca^{2+} levels by depleting intracellular stores and trigger the activation of nuclear Ca^{2+}-dependent endonuclease(s), facilitating the accompanying DNA fragmentation. Ca^{2+} may also exert its effects on thymocyte apoptosis via calmodulin-mediated activation of the Ca^{2+}-dependent protein phosphatase, calcineurin. GC-evoked apoptosis has been shown to generate ceramides via the sequential production or activation of the phosphoinosotide-specific phospholipase C, diacyl glycerol (DAG), protein kinase C, and acidic sphingomyelinase (aSMase). This process is dependent on GR, since RU 38486 can block the induction of aSMase production by dexamethasone (a synthetic glucocorticoid) in correlation with inhibition of apoptosis. This pathway is also linked to activation of the JNK/SAPK cascade, as demonstrated in stress-induced apoptosis of leukemic U937 cells. aSMase activation appears to mediate downstream activation of caspases, since inhibition of intermediate steps in the pathway blocks caspase activation in parallel with the prevention of apoptosis.

C. Protease Activation

Proteases are believed to be the ultimate mediators of irreversible changes in apoptosis. Members of the caspase family of aspartate-specific cysteine proteases have been implicated as the executioners of apoptosis in the lymphoid system. Caspase activity can be detected in immature double-positive thymocytes that are subject to apoptotic selection, but not in double-negative or mature thymocyte populations that are generally resistant to GC-evoked apoptosis, indicating that caspases act as apoptotic effectors. In recent years, mice generated by targeted disruption of individual caspase genes have provided important, although preliminary, information on their role in normal thymocyte development and selection. Mice deficient in caspase 1, 2, 3, or 9 exhibit normal development and distribution of thymocyte subpopulations, suggesting either that caspase activation is not essential for thymocyte selection or that there is redundancy in their action. Thymocytes from caspase 3 knockout mice are normal, whereas those from caspase 9 knockout mice exhibit a delayed death response to dexamethasone. In CEM cells, GC-evoked apoptosis is dependent on the activity of a member of the caspase 3 subfamily, but not on caspase 1 or its homologues, which are involved in Fas/Fas L-mediated apoptosis. Cell-surface differences in the recruitment of individual caspases have been reported in different subsets of isolated normal mouse T-lymphocytes and T-cell lines when triggered to undergo apoptosis by the same stimulus. Thus, great diversity exists in the recruitment of individual caspases by various apoptotic pathways. Other proteases, such as calpain, granzyme A, and proteosomes, also mediate apoptosis; however, details of their role in the process have not been forthcoming.

D. Mitochondrial Events

Loss of mitochondrial transmembrane potential and release of cytochrome c have been implicated as early events in apoptosis triggered by various agents; however, the precise mechanisms by which these events occur are still being debated. Also, in the case of GC-evoked lymphoid cell apoptosis, mitochondrial changes seem to be important for the activation of caspases; however, there is little information on the upstream events that trigger membrane permeability transition and loss of mitochondrial membrane potential. The release of cytochrome c is thought to recruit pro-caspases and adapter proteins to form an apoptosome, which activates initiator caspases via autocatalysis, setting on the caspase cascade that goes on to activate effector caspases, such as caspase 3, the final executioner in the process of cell death. The anti-apoptotic protein, Bcl-2, which acts via binding to the outer mitochondrial membrane and preventing cytochrome c release, is able to delay but not prevent GC-evoked lymphoid cell apoptosis, again suggesting that independent pathways may eventually lead to cell death.

VI. SUMMARY

Glucocorticoid hormones play an important role in normal tissue turnover and cellular homeostasis, in addition to their widely recognized contribution to the regulation of metabolic processes, ion transport, and stress responses. GC-induced cytostatic or lytic action in several tissues has been exploited for therapeutic intervention in diseases such as autoimmune disorders and leukemia. Paradoxically, this aspect of GC action in tissues such as bone is a contraindication for high-dose GC therapy. Studies on the lymphoid cell system have provided insights into the mechanisms of GC-evoked apoptosis. In modulating their action, GCs engage GRs, which may alter the expression of key genes either autonomously or via cross talk with other signaling pathways. GC-evoked apoptosis appears to require suppression

of proliferative genes, such as c-*myc* and *cyclin D3*, and up-regulation of proteins, such as $p27^{kipI}$ and c-Jun. DAG-, Ca^{2+}-, ceramide-, and aSMase-mediated signaling processes are activated and are believed to contribute to the apoptosis process. Indeed, there is increasing evidence that GCs trigger multiple, seemingly independent pro-apoptotic pathways in parallel, ultimately culminating in protease activation and the collapse of cellular machinery, which brings about irreversible apoptotic cell death. Much needs to be learned about the precise sequence of events that modulate GC-evoked apoptosis in lymphoid and other systems.

Glossary

apoptosis A genetically programmed process of cellular suicide characterized by cell shrinkage, membrane blebbing, nuclear condensation, and formation of membrane-bound cell fragments or "apoptotic bodies" that are engulfed by phagocytic cells.

avidity The total strength of an interaction between two multivalent surfaces.

caspases Family of aspartate-specific cysteine proteases that have been implicated in apoptosis.

glucocorticoid receptor Intracellular protein that binds with high specificity to glucocorticoids to form a hormone–receptor complex that is translocated to the nucleus and interacts with specific DNA sequences on target genes to elicit a transcriptional response.

major histocompatability complex (MHC) A set of membrane glycoproteins that present intracellular peptides to T-cells. MHC molecules are also involved in antigen processing and host defense.

osteoblasts Bone-forming cells derived from stromal mesenchymal cells and rich in alkaline phosphatase, collagens I and V, and other bone-specific proteins.

osteoporosis A progressive systemic skeletal disease characterized by low bone mass and deterioration of bone tissue, causing increased bone fragility and susceptibility to fracture.

T-cell selection The process that T-cells undergo during cell proliferation that eliminates potentially self-reactive cells and favors the survival of those cells that can recognize foreign antigens.

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