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Nuclear Hormone Receptors in T Lymphocytes

Review

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Among the numerous steroid and orphan nuclear receptors encoded within mammalian genomes, several are involved in regulating immune system functions. We review here recent studies on the glucocorticoid receptor and the orphan receptors Nur77 and RORγ. These molecules play key roles in the development and the effector functions of T lymphocytes.

Introduction

Steroid hormones have long been known to have a profound influence on the immune system. Their administration results in apoptosis of immature thymocytes and also in general immunosuppression characterized by compromised activation of antigen-specific lymphocytes, reduced cytokine production, and impaired function of antigen-presenting cells. Resection of the adrenals, the principal site for biosynthesis and systemic release of corticosteroids, results in thymic hyperplasia. These and other observations have led to the proposal that endogenous corticosteroids, through interaction with the glucocorticoid receptor (GR), a ligand-dependent transcription factor, have a key role in the selection of T cells during their development in the thymus (Ashwell et al., 2000). The role of GR and of its ligands in the development and function of the immune system has received much attention because of the therapeutic value of cortisone and related synthetic steroids in treatment of inflammation and malignancies. In recent years, several other steroid and non-steroid nuclear receptors have been shown to also have key functions in the development of the immune system, in shaping of the T cell repertoire, and in regulation of inflammatory responses. These include the vitamin D receptor, estrogen receptor, retinoic acid receptors (RAR), peroxisome proliferator-activated receptors (PPAR), and several orphan receptors that have no reported ligands (Alroy et al., 1995; Bissonnette et al., 1995; Moore et al., 2001; Staples et al., 1999). In this review, we will focus on the function in T cells of GR and several orphan nuclear receptors, members of the Nur77 family and RORγ, with a particular emphasis on their proposed diverse roles in the selection of the T cell repertoire.

To survive differentiation in the thymus and be exported to peripheral lymphoid organs, T cells must have appropriate interaction of their αβ T cell antigen receptor (TCR) with major histocompatibility complex (MHC) molecules loaded with endogenous peptide antigens (positive selection) (Jameson et al., 1995). More than 95% of immature double-positive (DP, $CD4^+CD8^+$) thymocytes undergo apoptosis. Most of these cells die by a process known as "death by neglect," due to sub-threshold or absent interaction of the TCR with host MHC/peptide complexes present on the thymic stromal cells. The signaling pathways involved in this form of apoptosis are poorly understood, and it remains unclear if their initiation is cell autonomous or triggered by extracellular factors. A small proportion of thymocytes undergo apoptosis triggered by high avidity interaction of the TCR with host MHC/peptide complexes, a process that purges the T cell repertoire of many potentially harmful self-reactive cells. The signaling events in this process, known as negative selection, are also poorly understood, but require intact TCR-associated signaling complexes. All nuclear receptors that will be discussed here have been implicated in regulation of thymocyte and T cell apoptosis. Nur77 and the related Nor-1 protein are proapoptotic factors that appear to have key roles in negative selection during development (Zhang et al., 1999). In experimental models, GR can also be shown to have proapoptotic activity, and clearly mediates corticosteroid-induced apoptosis in thymocytes, but its function under normal developmental and homeostatic conditions remains unclear. Whether GR is involved in promoting death by neglect or in setting the threshold for TCR-mediated selection signals has been a subject of considerable controversy, fueled most recently by studies with mutant mice that have altered or absent GR function (reviewed in Godfrey et al., 2001). In contrast, RORγ has antiapoptotic function in thymocytes and has been postulated to prevent premature death by countering constitutively active pathways that favor apoptosis (Littman et al., 1999).

The biochemical basis for nuclear receptor activity has been elucidated for several ligand-dependent factors. The receptors reside in either the cytoplasm or nucleus, and are activated upon binding of ligand to their C-terminal ligand-binding domain (LBD). They then bind to DNA as monomers, homodimers, or heterodimers in conjunction with other proteins (Tzameli and Moore, 2001; Weatherman et al., 1999). Steroid receptors, including GR, are often bound to cytoplasmic chaperones, and are subsequently released for transport to the nucleus upon binding of ligand (Pratt, 1997). Binding of ligand typically results in a conformational change that allows the LBD to interact with coactivators that have histone acetyltransferase activity, including members of the p160 and p300/CBP families (Glass and Rosenfeld, 2000; Weatherman et al., 1999). In the absence of ligand, DNA-bound nuclear receptors may fail to form active dimers, and/or they may be complexed with corepressor molecules, which in turn interact with histone deacetylases to repress transcription (Glass and Rosenfeld, 2000). In some cases, binding of ligand can interfere with recruitment of coactivators, and hence different

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ligands may either activate or inhibit the transcription of target genes under the direction of nuclear receptors (Tzameli and Moore, 2001). Production of ligand by cells within the microenvironment of the target cell can therefore influence the expression of genes regulated by nuclear receptors. However, for some orphan nuclear receptors, like Nur77 and RORγ, it is not clear if physiologically relevant ligands exist. The transcriptional activities of these proteins can be detected immediately following their expression, suggesting that their ligands might exist constitutively inside the cells or in serum or that they do not require ligand. Alternatively, these nuclear receptors may be modulated by ligands acting through receptor partners that form heterodimers.

The Role of Glucocorticoid Receptor in the Immune System

Glucocorticoids are commonly used as antiinflammatory and immunosuppressive drugs. In the body, they are produced by the adrenal gland and bind to the glucocorticoid receptor, one of the earliest identified members of the steroid receptor family. In contrast to RORγ and Nur77, which are mainly found in the nucleus, GR is held in the cytoplasm by heat shock proteins such as hsp70 and hsp90 (Pratt, 1997). Glucocorticoids cause a conformational change in the receptor, causing it to translocate to the nucleus where it exerts its function. GR binds as a homodimer to a DNA motif termed a glucocorticoid responsive element (GRE) and transactivates GRE-regulated genes. In addition to its transcriptional activities, GR can also exert negative influence by heterodimerizing and interfering with the activities of transcription factors like AP-1, NF-κB, CREB, and GATA-1. Thus, GR possesses two functions, one as a ligand-dependent transcription activating factor and the other as a ligand-dependent negative regulator of other transcription factors. The latter function is in most cases independent of DNA recognition by GR, and the receptor's zinc finger DNA binding domain is dispensable (Herrlich, 2001).

The function of GR in vivo has been assessed in several mouse lines bearing targeted mutations at the *GR* locus. GR-deficient mice died in the first minutes after birth, due to defective lung development (Cole et al., 1995). These mice also exhibited defective skin maturation, impaired erythrocyte progenitor proliferation, and other defects, suggesting that glucocorticoids are important for several aspects of mouse development. Interestingly, mice with a knockin mutation in the *GR* locus, which resulted in a dimerization-defective GR that was largely inactive for GRE-mediated transcription (termed $GR^{dim/dim}$), survived to adulthood with no apparent developmental defects, suggesting that the DNA binding function of GR is not required for early stages of mouse development (Reichardt et al., 1998). However, thymocytes from these mice were completely resistant to glucocorticoid-induced cell death. These data suggest that the transcriptional activity of GR is required to sensitize thymocytes to glucocorticoids, but that this activity may not be required for survival of the mouse.

The targets for GR-mediated transcription during steroid-induced thymocyte apoptosis remain to be identified, but it is known that caspase-9 is activated during this process. In mice lacking caspase-9, dexamethasone-induced thymocyte apoptosis was significantly impaired, which suggests that steroids activate the mitochondrial apoptotic pathway (Kuida et al., 1998). Consistent with this finding, dexamethasone-induced apoptosis was significantly reduced in transgenic mice expressing elevated levels of Bcl-2 or Bcl-xL in thymocytes (Chao et al., 1995). Treatment of thymocytes with glucocorticoids also results in a reduced level of the cell cycle inhibitor $p27^{kip1}$, and consequently in increased entry of cells into S phase (Gil-Gomez et al., 1998) (Figure 1). Because inhibition of cyclin-dependent kinases blocks thymocyte apoptosis, it has been proposed that cell cycle progression, in addition to the activation of caspases, is required for thymocyte apoptosis. Indeed, Bcl-2 and Bcl-xL can also block cell cycle progression by increasing levels of $p27^{kip1}$, through a mechanism that has not yet been elucidated. These antiapoptotic proteins may hence protect thymocytes from glucocorticoid-induced apoptosis by inhibiting the mitochondrial cell death pathway and by blocking cell cycle progression (Figure 1).

While GR clearly plays an important role in mouse development, its role in lymphocyte development remains controversial (Godfrey et al., 2001; Jondal et al., 2001). Although administration of glucocorticoids results in apoptosis of immature thymocytes, but not mature T cells, it is unclear whether endogenous corticosteroids have roles in normal T cell development in addition to their well-documented roles in responses to stress. Induction of thymocyte apoptosis by TCR ligation is inhibited by glucocorticoids (Ashwell et al., 2000) and, conversely, glucocorticoid-induced apoptosis can be inhibited by TCR-mediated activation of the ERK signaling pathway (Jamieson and Yamamoto, 2000). Based on these findings of mutual inhibition, Ashwell and colleagues have proposed that endogenous corticosteroids may regulate the thresholds for TCR-mediated positive and negative selection of thymocytes (Ashwell et al., 2000). They found that corticosteroids are produced by nonlymphoid cells in the thymus, and that blockade of glucocorticoid function in thymocytes, using either pharmacological agents or an antisense GR transgene, reduced the number of DP thymocytes and altered the T cell repertoire. In addition, T cells that normally undergo positive selection were found to undergo apoptosis, presumably because the normally weak positive selection signals become strong ones (negative selection) when the inhibitory effect of GR is reduced in these cells (Ashwell et al., 2000). However, the role of GR in normal T cell development has been challenged by several recent findings. First, the specificity of the antisense approach may be a problem, as another group reported an increase in the number of thymocytes in an independently derived transgenic line using the same promoter (*lck* proximal promoter) (Jondal et al., 2001). More importantly, total thymocyte cell numbers and the $CD4^+CD8^-$ and $CD4^-CD8^+$ subsets have been shown to be normal in $GR^{+/-}$ animals and in $GR^{dim/dim}$ mice (Cole et al., 1995; Reichardt et al., 1998). Recently, T cell development in fetal thymus was examined in $GR^{-/-}$ embryos (Purton et al., 2000). T cell subsets and cell numbers, as well as their ability to undergo

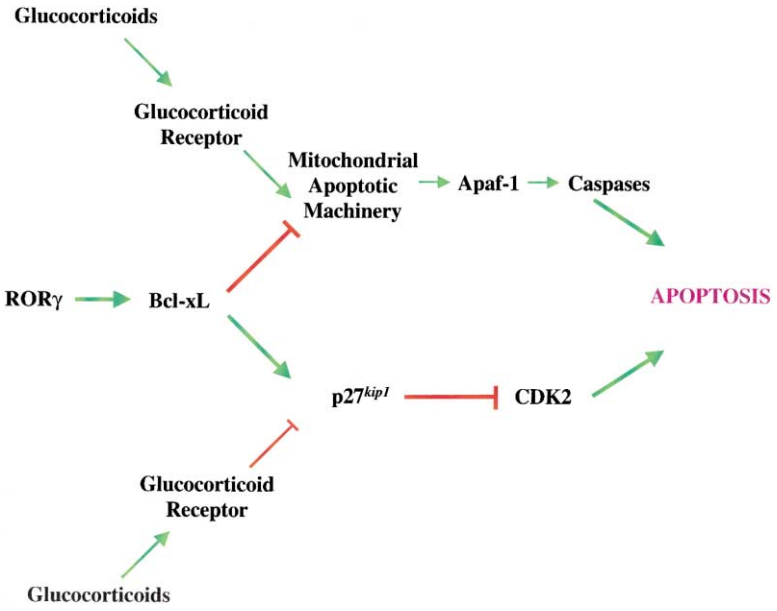

Figure 1. Proposed Mechanism of Action of Glucocorticoids and RORγ in Regulating Apoptosis of Double Positive Thymocytes

negative selection in response to anti-CD3/CD28 or superantigens were found to be largely normal. $GR^{-/-}$ thymocytes were completely resistant to glucocorticoid-mediated apoptosis, precluding the possible existence of another receptor with redundant function. These data cast doubt as to the function of GR during normal T cell development, at least during fetal stages. However, it remains possible that these conflicting results reflect differences in the strains of mice used or the effects exerted by the mutations or pharmacologic agents such as RU486 on cells other than thymocytes. It is also possible that in mice with dimerization-defective GR, glucocorticoids can still exert a developmental function in thymocytes unrelated to induction of apoptosis by GR target genes, but perhaps mediated by GR monomer in association with other transcription factors. Additional experiments, preferably with mice harboring thymocyte-specific mutations in GR, to eliminate possible effects in other cells, will need to be performed. Use of such mice to study negative selection and the role of TCR signaling will then help to settle the question of whether GR and glucocorticoids do play a central role in T cell development, particularly after the fetal stages.

In addition to their activity in thymocytes, glucocorticoids appear to have important functions in activation of the innate immune system and in antigen presentation (Moser et al., 1995). These effects likely account for the powerful anti-inflammatory effects of steroids. Although precise understanding of the mechanisms of immunosuppressive action of glucocorticoids is lacking, it is well established that ligand-dependent inhibition by GR of various transcription factors attenuates expression of inflammatory cytokines, which may result in skewing of the immune response (Herrlich, 2001; Vieira et al., 1998). In addition, GR can activate transcription of IκBα, which then serves to limit the function of NF-κB, a key transcriptional activator in both innate and adaptive immune responses (Auphan et al., 1995; Scheinman et al., 1995). Mice in which expression of GR was increased after introduction of a BAC transgene displayed not only enhanced susceptibility of thymocytes to steroids, but also increased resistance to lipopolysaccharide-induced septic shock (Reichardt et al., 2000). This result suggests that endogenous glucocorticoids normally play a role in limiting inflammation. Interestingly, in $GR^{dim/dim}$ mice, glucocorticoids were effective in limiting inflammatory responses, which includes activation of NF-κB, suggesting that GRE binding and activation of genes by GR are not required for its immunosuppressive functions and activation of IκBα transcription (Reichardt et al., 2001). Further studies are needed to determine the direct target genes that provide GR-mediated protection from inflammatory damage and to identify the cells involved in this process.

The Orphan Nuclear Receptor RORγ

The orphan nuclear receptor RORγ (also called TOR, RZRG, Thor, or NR1F3) was identified by virtue of its homology to RAR, and its mRNA was shown to be most abundantly expressed in the double-positive ($CD4^+CD8^+$) cells in thymus (Hirose et al., 1994; Ortiz et al., 1995). Subsequent studies revealed the predominant gene product in the thymus to be a closely related isoform, RORγt, generated by use of an alternative promoter within the second exon of the *RORγ* gene (resulting in a protein lacking the N-terminal 24 residues of RORγ) (He et al., 1998). RORγt was isolated using expression cloning aimed at identifying genes that inhibit activation-induced cell death (AICD) in a T cell hybridoma, but to date there has been no discernable difference in function between RORγ and RORγt. Forced expression of either isoform in T cell lines and hybridomas inhibited expression of IL-2 and Fas ligand, resulting in inhibition of both TCR-induced cell proliferation and cell death (He et al., 1998; Littman et al., 1999). RORγ inhibits NFAT-regulated transcription, which is required for TCR-mediated activation of both the *FasL* and *IL-2* genes. RORγ binds to the NFAT binding motif in the IL-2 enhancer, and thus competes with NFAT for binding to DNA (Littman et al., 1999). In response to its ligand,

vitamin D3 receptor (VDR) has similarly been shown to bind to the composite NFAT/AP-1 sites in the IL-2 and GM-CSF enhancers, thus explaining vitamin D3-induced transcriptional repression and inhibition of T cell proliferation (Alroy et al., 1995). Glucocorticoid activation of GR and retinoid activation of retinoid X receptor (RXR)-containing nuclear receptors have also been reported to inhibit FasL induction in T cells (Yang et al., 1995). Whether the transcriptional repression activity of RORγ also requires binding of a ligand or formation of a heterodimer is not yet known. Whereas VDR forms a heterodimer with RXR to mediate transcriptional activation upon binding to vitamin D3 responsive elements, it has been shown to repress transcription by binding as a monomer to the NFAT/AP-1 site. In the latter case, VDR monomer blocks binding of NFAT and, by direct interaction with AP-1, also inhibits its transactivating function (Towers et al., 1999). A requirement for both VDR and RXR in vitamin D3-mediated inhibition of NFAT binding has also been reported (Takeuchi et al., 1998). Although RORγ may also inhibit NFAT-responsive genes, this has not been demonstrated directly in untransfected cells or in vivo, and may not be relevant in mature T cells, which do not appear to express RORγ.

Biochemical studies on RORγ have been limited, and it remains unclear whether it functions as a monomer or as a homo- or heterodimer. When tethered to a heterologous Gal4 DNA binding domain, RORγ can activate transcription, through recruitment of coactivators of the p160 family (e.g., SRC-1) (Littman et al., 1999). It has not been determined whether this interaction is ligand independent and whether an N-terminal AF1 domain or the C-terminal domain confers the transactivating function. However, it appears that, similarly to the VDR, RORγ can function as a context-dependent transcriptional activator or repressor.

Analysis of mice in which the *RORγ* gene was disrupted revealed significant roles of its product(s) in both thymocyte development and in lymphoid organogenesis (Kurebayashi et al., 2000; Sun et al., 2000). In the thymus, there was a profound reduction in the number of double-positive thymocytes, and consequently also a reduction in mature thymocytes and T cells. This was due to a sharply increased rate of apoptosis of double-positive cells, which normally make up about 85% of total thymocytes. The phenotype was observed even in the absence of Fas-FasL interaction (in *gld/gld* mice) and there was no change in the FasL expression level in thymocytes of mutant mice, indicating that FasL is not a physiologically relevant target of RORγ in thymocytes. However, RORγ is required for expression of the antiapoptotic Bcl-xL molecule in thymocytes. Both Bcl-xL mRNA and protein, which are expressed at highest levels in DP thymocytes, were barely detectable in mice lacking RORγ. It is not yet known if Bcl-xL expression is under direct or indirect regulation by RORγ. Absence of RORγ also resulted in reduction of $p27^{kip1}$, and in subsequent unrestrained cell cycle progression of double-positive thymocytes, with a large proportion of the cells in S phase. This effect appears to be an indirect consequence of the reduction in Bcl-xL in mutant mice since thymocyte cell numbers, as well as $p27^{kip1}$ expression, were restored by forced expression of Bcl-xL (Sun et al., 2000). Studies have suggested that, in the thymus, activation of Cdk2, accompanied by entry of cells into S phase, is an obligate step in programming cells for apoptosis (Gil-Gomez et al., 1998). However, these studies relied on inducing apoptosis with corticosteroids, and it is unclear if physiological signals for inducing cell death (which are yet to be characterized) similarly require G1 to S phase progression. In mice that lack expression of $p27^{kip1}$, despite an increase in thymic cellularity and in S phase progression, there was no evidence of increased thymocyte apoptosis (Fero et al., 1996). It is thus possible that Bcl-xL regulates survival of double-positive thymocytes both by maintaining a sufficiently high level of $p27^{kip1}$ and by inhibiting mitochondria-dependent activation of the apoptotic pathway by proapoptotic members of the Bcl-2 family (Figure 1). Abrogation of both functions would then be required to observe premature apoptosis of these cells.

Immature DP thymocytes have been reported to undergo two waves of TCRα gene rearrangement and, hence, need to survive long enough to assure that if they are not selected after the first rearrangement, they have a second opportunity to be selected (Yannoutsos et al., 2001). It has been suggested that GR activation by endogenous glucocorticoids provides the proapoptotic stimulus in "death by neglect" of DP thymocytes. However, despite reduced dexamethasone-induced apoptosis in caspase-9 deficient mice, there was no evidence of thymic hyperplasia (Kuida et al., 1998). Further studies will need to be conducted, however, to determine if the lifespan of thymocytes is affected in the absence of GR or of caspase-9. It has been postulated that, by regulating Bcl-xL levels, RORγ controls the timing for survival and apoptosis of DP thymocytes (Sun et al., 2000). Thymocytes that undergo positive selection downregulate RORγ and Bcl-xL, but upregulate Bcl-2, which ensures their survival into mature T cells. Those cells that fail to be selected could die due to downregulation of RORγ or due to modulation in level of an RORγ ligand.

There has not yet been any demonstration of target genes that are directly regulated by RORγ in vivo. RORγ was shown to bind to a putative consensus motif in the TEA element upstream of the Jα cluster in the *TCRα* locus (Villey et al., 1999). This element is required for rearrangement of Vα segments to the 5′ Jα segments. Such rearrangements are favored early in development, and subsequent receptor editing in DP thymocytes is thought to involve rearrangements to Jα segments located at 3′ locations (Yannoutsos et al., 2001). It has therefore been proposed that RORγ may have a role in TEA element function, regulating the early wave of rearrangements within the TCRα locus. Analysis of the TCR repertoire in RORγ-deficient mice has not yet been reported.

RORγ has also been shown to be required for the development of lymph nodes and Peyer's patches (Kurebayashi et al., 2000; Sun et al., 2000). In its absence, there was loss of a population of embryonic cells with the phenotype $CD4^+CD3^-IL\text{-}7R\alpha^+\alpha4\beta7^+$, which have been proposed to be progenitor cells in lymph node development (Sun et al., 2000). A similar phenotype of lymphoid organ deficiency, including the loss of this specialized cell population, has been observed in mice lacking the HLH inhibitory transcription factor Id2 (Yokota et al.,

1999). Both Id2 and RORγ are normally expressed in these putative progenitor cells, which also express lymphotoxin (LT)-αβ heterotrimers and TRANCE/RANKL, members of the TNF family (Kim et al., 2000; Mebius et al., 1997). Whereas LT and TRANCE are required for development of splenic follicles as well as lymph nodes and Peyer's patches, the transcription factors RORγ and Id2 are not required for development of the spleen. This is consistent with a central role of splenic B cells in production of LT, which is required for the formation of the follicular dendritic network (Fu and Chaplin, 1999). The LT-expressing $CD4^+$ embryonic cells may therefore have a similar function in regulating the differentiation of the early lymph nodes and Peyer's patches. Remarkably, RORγ is not required for the differentiation of nasal associated lymphoid tissue (NALT), whose organization closely resembles that of Peyer's patches (Harmsen et al., 2002). These findings indicate that the diverse lymphoid organs have different mechanisms of early differentiation. All require expression of lymphotoxin heterotrimers and triggering by the LTβ receptor of RelA-containing NF-κB in cells reported to be radioresistant stromal cells (Alcamo et al., 2002), but different LT^+ cell types migrate to specific sites to direct development of each organ.

The Nur77 Family of Orphan Steroid Receptors

Nur77 (also called TR3, NGFI-B, NAK1, and 8 other aliases) is an inducible orphan nuclear receptor first identified as an immediate early serum-induced gene (Zhang et al., 1999), and subsequently as a gene induced by T cell receptor (TCR) signaling in T cell hybridomas and thymocytes (Liu et al., 1994; Woronicz et al., 1994). In contrast to GR and RORγ, which are expressed in resting cells and whose expression does not change appreciably in activated cells, Nur77 transcription is controlled by external stimuli. Nur77 mRNA is upregulated in both B and T lymphocytes following antigen receptor ligation, in fibroblasts in the presence of serum, and in nerve cells following their stimulation with nerve growth factor (NGF) or in response to seizures and mechanical lesions (Liu et al., 1994; Mittelstadt and DeFranco, 1993; Woronicz et al., 1994). Interestingly, the kinetics of Nur77 induction in response to stimulation by serum, NGF, or phorbol ester is transient and differs from that in T cells activated by TCR, in which induction is long lasting. The functional significance of differential kinetics of Nur77 induction is not well understood, but steady expression of Nur77 protein may induce apoptosis while transient expression does not. Indeed, constitutive expression of Nur77 in thymocytes and in several cell lines leads to apoptosis (Calnan et al., 1995; Li et al., 2000; Masuyama et al., 2001; Weih et al., 1996). However, the fact that Nur77 and its family members, Nor-1 and Nurr1, are expressed as immediate early genes in different cell types following various stimuli, suggests that they play a role in a diverse set of biological functions. Indeed, Nurr1-deficient mice lack midbrain dopaminergic neurons and die soon after birth (Zetterstrom et al., 1997).

Similar to other steroid receptor family members, Nur77 consists of an N-terminal AF1 transactivation domain, a DNA binding domain with two zinc fingers, and a C-terminal ligand binding domain. The other family members, Nor-1 (also called MINOR and 3 other names) and Nurr1 (also called NOT, RNR1, TINOR, and TINUR), bear more than 90% homology in their DNA binding domains but are divergent in their N- and C-terminal regions (Cheng et al., 1997). All three family members can bind to a consensus NBRE sequence (AAAGGTCA) as monomers or to a palindromic DNA binding motif (NurRE, $TGATATTTX_6AAATGCCA$) as homodimers (Philips et al., 1997). Expression of Nur77, Nor-1, or Nurr1 alone is sufficient to activate NBRE or NurRE-directed transcriptional activities, suggesting that the Nur77 family members might be "constitutive" orphan steroid receptors that do not require ligands for activation. In addition to its transcriptional function, Nur77 can also act as a modulator of transcription for other orphan steroid receptors. For example, Nur77 and Nurr1 can heterodimerize with RXR in the presence of retinoids and modulate the activities of a subclass of retinoid responsive elements (Forman et al., 1995; Perlmann and Jansson, 1995). Nur77 has also been reported to bind and alter the activity of COUP-TF, an orphan steroid receptor thought to negatively regulate the activation function of vitamin D receptor, RAR, RXR, and PPARs (Wu et al., 1997). Conversely, because of its interaction with RAR and RXR, Nur77 activity can be modulated by natural and synthetic retinoic acids to influence cell survival. All-*trans* or 9-*cis*-retinoic acids, which block AICD, also inhibit the transcriptional activity of Nur77 (and expression of FasL) in T cells (Kang et al., 2000; Toth et al., 2001). In contrast, CD437, a RARγ-specific agonist, induces Nur77 expression through an unknown mechanism and enhances Nur77-mediated NBRE activity (Li et al., 1998; Toth et al., 2001). CD437 was shown to induce growth arrest and apoptosis of a variety of cancer cell lines in a p53-independent fashion. This apoptotic activity was reported to be mediated through Nur77 (Li et al., 2000, 1998).

Nur77 is a phosphoprotein that typically migrates between 65 and 75 kDa. Phosphorylation of Nur77, which results in its predominant localization in the cytoplasm, can be carried out by several kinases. These include members of the MAP kinase family and the protein kinase Akt (Figure 2). Akt phosphorylates Nur77 in its DNA binding domain, resulting in reduced Nur77 DNA binding activity (Pekarsky et al., 2001). Overexpression of Akt reduces activation-induced cell death of T cell hybridomas and can partially protect against wild-type Nur77-mediated apoptosis in Rat1a fibroblasts (Masuyama et al., 2001). In light of the fact that not all cell lines are susceptible to Nur77-induced apoptosis, it will be interesting to see if the activity of Akt correlates with resistance of cells to Nur77-mediated cell death.

In PC12 nerve cells, phosphorylation of Nur77 in its N-terminal region by members of the MAP kinase family regulates the ability of Nur77 to be exported to the cytoplasm in response to NGF (Katagiri et al., 2000). Stimulation of PC12 cells by NGF results in upregulation of Nur77 protein and in its phosphorylation and subsequent translocation to the cytoplasm. Interestingly, RXR is also transported to the cytoplasm along with Nur77 through heterodimerization. Nur77 contains three nuclear export signals located in the "ligand binding" domain that, when mutated, cause Nur77 to remain in the

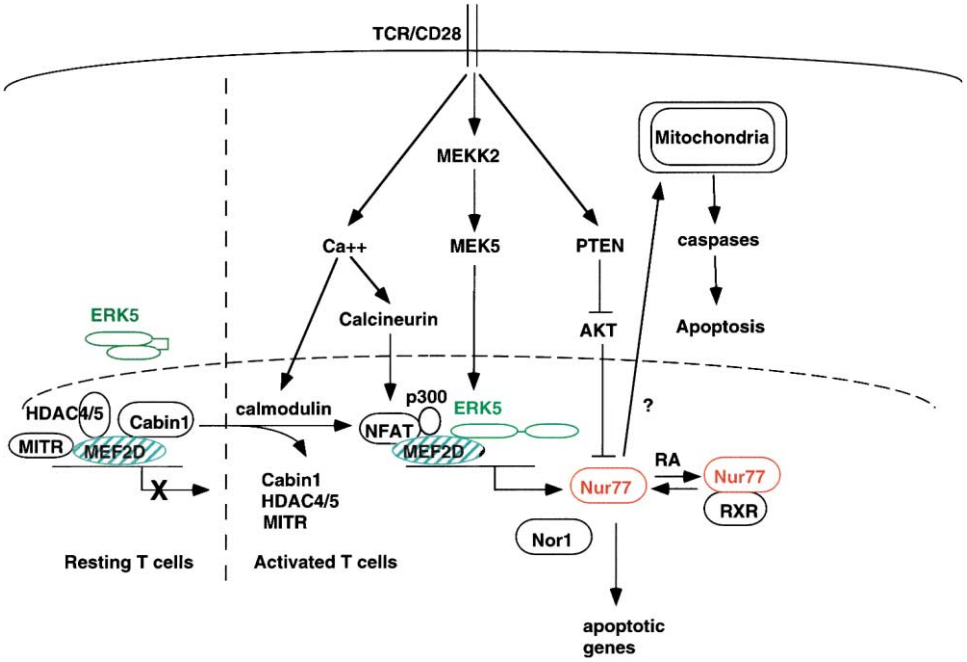

Figure 2. A Schematic Diagram of Nur77 Signal Transduction

nucleus despite the presence of NGF (Katagiri et al., 2000). Mutating the MAP kinase serine phosphorylation site of Nur77 to alanine also abolishes its growth factor-induced export from the nucleus. These data suggest that NGF stimulation results in phosphorylation of Nur77, thus exposing the export signals within the C-terminal ligand binding domain and causing translocation of Nur77 to the cytoplasm. Whether a similar situation occurs in T cells or other immune cells has not been explored.

In T cells, Nur77 transcription triggered by TCR signaling can be inhibited by cyclosporin A, suggesting that calcineurin plays an important role in its regulation. Microarray analyses have confirmed that transcripts for Nur77 family members are rapidly upregulated in mature T cells stimulated with phorbol ester and ionomycin, whereas they are not induced in T cells derived from severe-combined immunodeficient patients with calcium flux defects (Feske et al., 2001). Similarly, Nur77 and Nor-1 mRNAs/proteins were readily detectable in thymocytes as early as 30 min after stimulation with phorbol ester and ionomycin (Woronicz et al., 1995). Maximal induction of Nur77 in $CD4^+CD8^+$ thymocytes requires a signal through TCR and additional stimulation through other cell surface proteins (Amsen et al., 1999). In contrast, molecules associated with positive selection or maturation of single positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes, Egr-1, ERK2, and CD69, are not affected by the costimulatory activities. While the role of costimulatory molecules in negative selection in vivo has not been clearly established, apoptosis of DP thymocytes in vitro requires combined signals from the T cell receptor and additional molecules, including CD28 (Kishimoto and Sprent, 1999). Consistent with these data, transgenic mice expressing dominant-negative Nur77 were defective in the process of negative selection but positive selection proceeded normally (Calnan et al., 1995; Zhou et al., 1996). Dominant-negative Nur77 protein, however, has been shown to inhibit the activities of all Nur77-related molecules (Cheng et al., 1997). As the kinetics of Nor-1 expression in activated T cells mirrors that of Nur77, Nor-1 is likely to share function with Nur77 during T cell development (Cheng et al., 1997). Similar to Nur77, constitutive Nor-1 expression leads to massive thymocyte cell death (Cheng et al., 1997). Functional redundancy between Nur77 and its related proteins might explain the complete lack of a phenotype in Nur77-deficient mice (Lee et al., 1995). Recently, mice deficient in PTEN, a known inhibitor of Akt, were shown to be defective in negative selection (Suzuki et al., 2001). Although not demonstrated directly, this is consistent with an inhibitory role of Akt in the regulation of Nur77, as discussed above. The function of Nur77 and its family members has not yet been assessed in mature T cells.

Nur77 transcription is regulated by a complex web of transcription factors and other proteins (Figure 2). Using promoter deletional analysis, two MEF2 binding sites were identified as TCR-regulated transcriptional elements in the Nur77 promoter region (Woronicz et al., 1995). Interestingly, these MEF2 sites lie upstream and are separated from transcriptional elements that are responsive to serum and NGF stimulation (Williams and Lau, 1993; Yoon and Lau, 1993). There are four MEF2 family members: MEF2A, MEF2B, MEF2C, and MEF2D. These transcription factors bear homology to the MADS-box proteins. Other than MEF2C, whose expression is restricted to muscle and B cells, all other MEF2 family members are expressed widely. In T cells, MEF2D is the dominant MEF2 family member. Expression and phosphorylation of the MEF2 proteins do not change upon TCR stimulation. Recently, several repressors and acti-

vators were found to associate with MEF2D. In resting cells, Cabin-1, a calcineurin-interacting repressor protein, was found to associate with MEF2D (Youn and Liu, 2000; Youn et al., 1999). Overexpression of Cabin-1 in T cell hybridomas inhibited Nur77 induction and AICD (Youn and Liu, 2000; Youn et al., 1999). In vivo, deletion of the Cabin-1 C-terminal MEF2-interacting domain did not affect apoptosis or Nur77 expression but enhanced cytokine production (Esau et al., 2001). The lack of an absolute requirement for Cabin-1 in repressing MEF2D-dependent Nur77 expression may reflect the redundant function of other MEF2D interacting repressors. These include the histone deacetylase HDAC4 (Miska et al., 1999) and the HDAC-like protein MITR (Sparrow et al., 1999).

MEF2D is a weak transcription factor, and dissociation of various repressors from MEF2D in activated T cells is not sufficient to activate Nur77 transcription. NF-ATc1, through a DNA-independent mechanism, can coactivate MEF2 DNA elements (Blaeser et al., 2000; Youn et al., 2000). In addition, ERK5, a kinase with a unique C-terminal transcriptional activation domain, can also associate with MEF2D and deliver a powerful transcriptional activating activity (Kasler et al., 2000). ERK5 is the fourth class within the MAP kinase family and is activated by the upstream MEK5 kinase (Zhou et al., 1995). MEK5 is in turn activated by MEKK2 or MEKK3 kinases, depending on the stimuli (Chao et al., 1999). ERK5 activation, achieved by coexpression of an activated form of MEK5 by expression of a truncated protein containing only the C-terminal region, can stimulate Nur77 transcription in the absence of any TCR signaling (Kasler et al., 2000). Furthermore, a mutant form of ERK5 that lacks the C-terminal transcriptional activation domain acts as a dominant-negative protein that suppresses induction of Nur77 transcription (Kasler et al., 2000). Thus, coordinated regulation through release of repressors and simultaneous activation of coactivators constitutes a mechanism whereby T cells can activate Nur77 transcription to a high level in a relatively short time.

Few downstream genes for Nur77-mediated transcription have been identified. Although the expression levels of FasL and CD30 are elevated in Nur77 transgenic mice (Weih et al., 1996; Zhang et al., 1999), Nur77-mediated apoptosis is still intact in *gld/gld* (FasL mutant mice) or $CD30^{-/-}$ mice, precluding FasL and CD30 as the major downstream effectors for Nur77 in thymocytes (Chan et al., 1998; Zhang et al., 1999). Consistent with this notion, negative selection of $CD4^{+}CD8^{+}$ (DP) thymocytes is intact in Fas- or CD30-deficient mice (DeYoung et al., 2000; Singer and Abbas, 1994). In thymocytes, Nur77 apoptotic activity correlates with its transcriptional function (Kuang et al., 1999). Truncation of the Nur77 N-terminal transactivation region results in a protein with dominant-negative activity. A small deletion of its C-terminal ligand binding domain abolishes its transcriptional and apoptotic activities. Surprisingly, complete truncation of the C-terminal region leads to potent Nur77 transcriptional activity, and expression of this derivative in mice leads to massive thymocyte apoptosis.

Recently, Nur77 has also been reported to initiate apoptosis by translocation to the mitochondria, followed by the release of cytochrome c into the cytoplasm (Dawson et al., 2001; Li et al., 2000). The latter event causes activation of Apaf-1 and caspase-9, followed by cleavage of pro-caspase 3, leading to apoptosis (Li et al., 1997). These experiments, done in prostate and lung cancer cell lines, showed that the Nur77 DNA binding domain is dispensable for this process. These data suggest that Nur77 transcription is not required for apoptosis under these conditions. How Nur77 is translocated to mitochondria, however, is not clear, as Nur77 has no mitochondrial targeting sequence. Similarly, it is not understood how Nur77 causes cytochrome c release into the cytoplasm. In T cell hybridomas, Nur77 is mostly localized to the nucleus. Interestingly, overexpression of Bcl-2 does not block Nur77-mediated apoptosis in T cells (Zhang et al., 1999), suggesting that either Nur77 transcription plays a more prominent role in initiating apoptosis specifically in T cells or that Nur77 initiates a Bcl-2-independent mitochondrial apoptotic pathway. Additional experiments are necessary to identify the Nur77 downstream target genes responsible for regulating T cell apoptosis and to elucidate the mechanisms of Nur77 localization to mitochondria in different cell types.

Conclusions

We have reviewed recent advances in understanding the role of three members of the nuclear hormone receptor superfamily in development and regulation of the immune system. Although all of these receptors have been implicated as having key roles in promoting either thymocyte survival or apoptosis, their in vivo functions have not yet been definitively characterized. Gene inactivation studies have not yet identified clear roles in thymocytes or in mature T cells for GR or for members of the Nur77 family, particularly Nor1, and generation of tissue-specific gene targeted mice will be necessary to address their roles in the immune system. Targets for different functions of these nuclear receptors also need to be identified. For the orphan steroid receptors, it is still not clear if physiological ligands exist. Nevertheless, identification and application of synthetic agonist or antagonist ligands could prove useful in clinical settings to modulate human immune responses and may lead to useful therapeutic applications.

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