Characterization of the Myc collaborating oncogenes Bmi1 and Gfi1
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Citation for published version (APA):
Chapter 1

Introduction

Transcriptional control of apoptosis in lymphocytes

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At many occasions during their lifespan, lymphocytes will be subjected to signals that decide for life or death. Cytokine signaling, antigen receptors-mediated selection, or activation of members of the TNF receptor superfamily often impose these decisions. In the last few years various important players and executors of the active death machinery have been identified, including many Bcl-2 and caspase family members. However, information about the transcriptional control of programmed cell death in B and T lymphocytes, is still limited and remains to be revealed. Here we summarize our current state of knowledge on a broad collection of transcriptional regulators able to alter the balance between cell death and survival in lymphocytes.

[Key words: apoptosis; B lymphocytes; negative selection; thymocytes; transcription factors]

The process of programmed cell death or apoptosis plays a central role in regulating lymphocyte development and homeostasis in mammals. Mainly three different forms of signals decide over life and death in B and T lymphocytes. One form of survival signaling is mediated through the action of cytokines, which not only control differentiation and proliferation, but clearly also mediate rescue from cell death. Cytokines like interleukin-3 (IL-3), IL-4 and IL-7 promote cell survival in T and B cells (Boise et al. 1995; Vella et al. 1997; Vella et al. 1998; Venkataraman et al. 1998; Kuribara et al. 1999). On the other hand certain cytokines can also sensitize lymphocytes for cell death like IL-2 (Refaeli et al. 1998; Van Parijs et al. 1999) and TGFβ (Wahl et al. 2000).

The second mechanism involves proof-reading of the antigen receptors, which results in the largest proportion of cell death during B and T cell development (Strasser 1995). As much as 75% of the B lymphocytes in bone marrow and 97% of the thymocytes will die because they have nonproductive immunoglobulin and T cell receptor gene rearrangements or express an inadequate antigen receptor on their cell surface. B cells express antibodies as cell membrane receptors with single antigen specificity. B cells are selected in the bone marrow on the basis of the affinity antibodies: cells with high affinity for epitopes derived from 'self' are eliminated (Lu and Osmond 2000). Mature B lymphocytes leave the bone marrow and populate spleen, lymph nodes and the gut-associated lymphoid tissue. Once activated by an antigen, B cells undergo a second round of selection in the follicles of secondary lymphoid organs, after which they mature into plasma cells and subsequently recirculate to the bone marrow (Osmond 1993).

Pro-T lymphocytes emigrate from the bone marrow into the thymus, where they further mature and become subjected to two separate rounds of thymocyte selection. The first one occurs at the pre-T cell stage and involves selection of a functional T cell receptor (TCR) β chain in the absence of ligand binding (β-selection) (von Boehmer et al. 1999). At the second stage, CD4+CD8+ double positive (DP) T cells will undergo positive and negative selection of the complete T cell antigen receptor, which depends on the affinity of the TCR for self major histocompatibility (MHC) antigens (Saito and Watanabe 1998; Mariathasan et al. 1999). MHC class I and II antigens are molecules that sample fragments from foreign and self-peptides, respectively. Each MHC class I or II protein presents a different fragment. T cells with a high affinity for self-MHC molecules are eliminated (negative selection); in the complete absence of any MHC-TCR
interaction thymocytes will die by neglect. Only T cells that produce a functional TCR with appropriate avidity/affinity for self-peptide-MHC molecules will be positively selected, leave the thymus and populate the secondary lymphoid organs.

The third system that controls lymphocyte survival signaling involves the large tumor necrosis factor (TNF)-receptor superfamily (Baker and Reddy 1998; Gravestein and Borst 1998; Screaton and Xu 2000). The TNFRs can be divided into two groups, based on the presence or absence of a cytoplasmic death domain (DD). DD-lacking receptors include CD27, CD30, CD40, TNFR2/CD120b, RANK, OPG and low affinity nerve growth factor receptor. The DD-containing receptors include Fas/Apo/CD95, TNFR1/CD120a, DR3/TRAMP/Apo3, DR4/TRAIL-R1 and DR5/TRAIL-R2/KILLER. While Fas specifically mediates apoptosis, TNFRs mediate cell survival as well as cell death, through the differential activation of the transcription factor NK-κB (Beg and Baltimore 1996). Signaling through TNFR2 can induce cell death, by recruitment of TRAF2 and in the presence of the protein kinase RIP, whereas in the absence of RIP TNFR2 activates NF-κB and promotes survival (Pimentel-Muninos and Seed 1999).

Whereas lymphocytes on one hand are largely directed by the signals they receive from outside the cell via the different kinds of cell surface receptors, genetic control may alter the outcome of these receptor signaling cascades or bypass the requirement for specific signals. Transcriptional regulators are able to control apoptosis by acting upstream of the death receptors or cytokine signaling pathways, by altering the expression levels of individual proximal (ligand/cytokine or receptor) or distal (Bcl-2 family members) components of such signaling cascades. For instance, regulation of FasL expression has been studied extensively, and appears to be induced by a variety of transcription factors, including p53, c-Myc, NFAT, Egr-2, Egr-3, NF-κB, IRF-1, Sp-1 and ALG-4 (Latinis et al. 1997; Kasibhatla et al. 1999; Lacana and D'Adamio 1999; Li-Weber et al. 1999; Mittelstadt and Ashwell 1999; Brunner et al. 2000; Chou et al. 2000; Chow et al. 2000; Rengarajan et al. 2000). Alternatively, transcriptional regulators may be the physiological end targets of specific signaling cascades able to modify cell death versus survival, like NF-κB. Different expression levels could alter the outcome of the intended signal delivered to the cell.

We will review the biological activities relevant for lymphoid development and survival of several transcriptional regulators able to direct apoptosis control in lymphocytes.

p53/p73

The p53 gene encodes a transcriptional activator with a sequence specific DNA-binding domain, and is the most frequently mutated gene in human cancers (Hollstein et al. 1991; Levine et al. 1991; Greenblatt et al. 1994). Analysis of the tumors from Li-Fraumeni patients, which carry a germline mutation in the p53 gene, reveals that in most cases there is loss of heterozygosity (LOH) at the p53 locus, arguing that p53 has growth suppressing activity (Malkin et al. 1990; Iavarone et al. 1992; Srivastava et al. 1992). Similarly, mice heterozygous mutant for p53 display a strong predisposition to cancer, including thymic lymphomas, soft tissue sarcomas and osteosarcomas (Harvey et al. 1993; Jacks et al. 1994; Purdie et al. 1994). However, a large proportion of the tumors from the p53∗/mice retains the intact, functional wild type allele, indicating that a mere reduction in p53 levels may be sufficient to promote tumorigenesis (Venkatachalam et al. 1998).

Tumor suppressor p53 has been nominated "guardian of the genome", since it is a critical component of cellular mechanisms that are activated upon genotoxic stresses, like DNA damage or hypoxia, which allow maintenance of genomic integrity in part by arresting cell-cycle progression or inducing apoptosis (Levine 1997; Amundson et al. 1998; Giaccia and Kastan 1998; Somasundaram 2000). Levels of p53 in the cell must be closely controlled to maintain cell viability. Regulation of cellular localization, active and inactive protein conformations and protein stabilization all contribute to this control (Kubbutat and Vousden 1998).

One important pathway regulating p53 function involves control by Mdm2 and p19ARF.
The tumor-suppressor protein p19\(\alpha\)-f11A (Oliner et al. 1993; Picksley et al. 1994; Chen et al. 1996) exhibits negative feedback with respect to p53 function (Momand et al. 1992; Barak et al. 1993; Oliner et al. 1993), and Mdm2 is a critical cellular inhibitor of p53 function, thereby exhibiting negative feedback with respect to p53 function (Momand et al. 1992; Barak et al. 1993; Oliner et al. 1993; Picksley et al. 1994; Chen et al. 1996). The tumor-suppressor protein p19\(\alpha\)fp interacts with Mdm2 and sequesters it into the nucleoli (Zhang et al. 1998; Weber et al. 1999; Weber et al. 2000), thereby mediating stabilization of p53.

Thymocytes lacking \(p53\) expression are resistant to apoptotic cell death following treatment with ionizing radiation and etoposide, but retain normal sensitivity to glucocorticoids, calcium ionophores, anti-CD95/Fas, or T cell receptor-induced apoptosis (Lowe et al. 1993; Boehme and Lenardo 1996). Remarkably, mitogen-stimulated T cells and cycling T lymphoma cells from \(p53^{−/−}\) mice still undergo apoptosis after irradiation or genotoxic drug treatment, which is inhabitable by Bcl-2 (Strasser et al. 1994). This indicates that there is a \(p53\)-independent pathway that fulfills an essential role in protecting cycling T cells against genotoxic stress. T cell development is not disturbed in \(p53^{-/}\) deficient mice, and there are no indications that basal apoptosis levels in thymocytes are altered. On the other hand, \(p53\) clearly reduces apoptosis in pro-B cells with concomitant expansion of the pro-B cell population, without affecting cell death in pre-B and B lymphocytes (Lu et al. 1999). There are however no indications yet that \(p53^{-/}\) mice have an increased risk to develop (pro-) B cell tumors. B cell specific inactivation of \(p53\) expression using conditional mutant mice, may address this aspect in the near future.

Several \(p53\) target genes, implicated in apoptosis control, have been identified, including \(bax\) (Miyashita and Reed 1995), \(bcl-x\) (Zhan et al. 1996), \(fasp\) (Owen-Schaub et al. 1995), \(IGFBP3\) (Buckbinder et al. 1995), \(PIG1\)-\(PIG14\) (Polyak et al. 1997), \(PAG608\) (Israeli et al. 1997), \(DR5\) (Wu et al. 1997), \(p85\) (Israeli et al. 1997), \(TRID\) (Sheikh et al. 1999), \(TRUNDD\) (Meng et al. 2000), \(PIDD\) (Lin et al. 2000), \(p53AIPI\) (Oda et al. 2000b), \(PERP\) (Attardi et al. 2000) and \(Noxa\) (Oda et al. 2000a). Although induction of \(bax\) expression upon \(p53\) activation is well established, \(bax\) is apparently not required for \(\gamma\)-radiation-induced apoptosis, since this form \(p53\)-dependent cell death still occurs in thymocytes of \(bax^{−/−}\) thymocytes (Knudson et al. 1995). Conversely, transgenic \(bax\) expression does not restore DNA damage-induced apoptosis in \(p53^{−/−}\) T cells (Brady et al. 1996). Therefore other \(p53\) target genes induced upon DNA-damage, like the pro-apoptotic Bcl-2 family member \(Noxa\), may mediate genotoxic stress-induced apoptosis in (lymphoid) cells.

Interestingly, there is now more compelling evidence that \(p53\) has also a role in mediating early T cell survival signaling. In recombinaisactivating gene (Rag)-deficient mice, antigen receptor rearrangements will not occur and T cell differentiation arrests at the pro-T3 CD44/CD25+ CD4/CD8+ double negative (DN) stage (Mombaerts et al. 1992). In the absence of pre-TCR signaling, pro-T3 cells have a limited lifespan of ~3-4 days, after which they will die. In \(p53^{−/−}\) mice, T cell development progresses to the CD4+CD8+ DP stage, but without inducing pre-T cell expansion (Jiang et al. 1996). In \(scid/DNA-PK\)-mutant mice, lymphocyte development arrests at the same pre-T3 stage, because the mutation in DNA-PK produces ‘broken’ V(D)J coding ends preventing assembly of functional receptor genes. Rescue of thymocyte development in \(p53^{−/−}scid\) mice is however more prominent than in \(p53^{−/−}rag^{−/−}\) mice, with also increased thymocyte cell numbers and restoration of TCRβ rearrangements (Bogue et al. 1996; Guidos et al. 1996; Nacht et al. 1996). In addition, \(p53\) rescues cell death in DN pre-T cells that is dependent on Rho- (Costello et al. 2000) or CD3γ-signaling (Haks et al. 1999), although thymic cellularity is only restored in CD3δ-deficient mice. Therefore, it seems that \(p53\) controls some aspects of survival signaling downstream of pre-TCR that could depend on transcriptional regulation of critical death receptors, such as DR5 (Wu et al. 1997; Wu et al. 1999;
Takimoto and El-Deiry (2000) or PIDD (Lin et al. 2000), by p53, since DN-FADD also bypasses the requirement for pre-TCR signaling (Newton et al. 2000).

Several p53 family members have recently been identified, including p40 (Trink et al. 1998), p51 (Osada et al. 1998), p63 (Yang et al. 1998), and p73 (Jost et al. 1997; Kaghad et al. 1997). Within the conserved domains, they all have considerable homology with p53. The p53 target gene p21<sup>"ramp"</sup> is induced by p51, p63 as well as p73, and they also elicit apoptosis when overexpressed. Recent data show that p73 is critical in mediating TCR-triggered activation induced cell death (AICD) (Lissy et al. 2000), and likely acts in a linear pathway with E2F-1. At present it is not clear at which level specificity is generated between E2F-1-mediated induction of p53 and p73 in the context of TCR-activation. One option is that both genes are equally induced but p73 controls expression of a different (set of) target genes important in mediating clonal deletion. Alternatively, p73 may display different protein stability characteristics upon TCR-signaling. Interestingly, p73α is not targeted for degradation upon binding to Mdm2 or human papilloma-virus E6 protein (Balint et al. 1999), providing evidence that different signaling cascades may regulate p53 and p73α protein stability.

**NF-κB/Rel**

The nuclear factor (NF)-κB-like transcription factors have been shown to regulate apoptosis in response to a variety of cytotoxic signals and agents (Baueuerle and Baltimore 1996; Sonenshein 1997b). In mammals there are five distinct subunits. NF-κB1 (p50) and NF-κB2 (p52) only consist of the Rel homology domain and lack intrinsic transcriptional activation properties, whereas Rel, RelA (p65) and RelB have distinct transactivation domains (Baueuerle and Henkel 1994). The major proportion of Rel/NF-κB is in most cell types sequestered in the cytoplasm in an inactive form through association with regulatory IκB proteins (Finco and Baldwin 1995; Verma et al. 1995). A broad range of stimuli promote nuclear translocation of cytoplasmic Rel/NF-κB complexes by a mechanism that involves the activation of an IκB kinase complex (Gerondakis et al. 1998). This phosphorylates specific amino-terminal serine residues within the various IκB isoforms, thereby targeting IκB for ubiquitin-dependent proteosome-mediated degradation.

One prominent role of NF-κB is its ability to prevent TNF-receptor signaling induced cell death (Beg and Baltimore 1996). This also includes protection against Fas/Apol-mediated cell death in T lymphocytes (Dudley et al. 1999; Rivera-Walsh et al. 2000). NF-κB seems also to act as a selective survival signal in pre-T cell development, and may substitute for absence of Bcl-2 expression at the transition of pro-T3 to CD44<sup>CD25</sup> DN T cell stage (Voll et al. 2000).

Several different transcriptional targets of Rel/NF-κB, which fulfill anti-apoptotic functions, have been identified. These include TNF receptor-associated factors, like TRAF1 and TRAF2, and cellular inhibitors of apoptosis proteins c-IAP1, c-IAP2 (Wang et al. 1998), xIAP (Stehlik et al. 1998), and ch-IAP1 (You et al. 1997), all of which participate in protecting cells against TNF-α-induced cell death. Other Rel-regulated prosurvival genes are bcl-2 homolog A1, which is required to prevent antigen receptor ligation-induced cell death in B cells (Grumont et al. 1999), bcl-x<sub>L</sub> (Chen et al. 2000; Khoshnan et al. 2000) and c-myc, which is implicated in preventing anti-IgM-induced apoptosis in the immature B cell line W231 (Arsura et al. 1996; Sonenshein 1997a), as well as protecting T lymphocytes against glucocorticoid-induced apoptosis (Thulasi et al. 1993; Wang et al. 1999).

However, NF-κB can also promote apoptosis under different circumstances. NF-κB has been identified as a mediator of p53-dependent apoptosis (Ryan et al. 2000). Inhibition or loss of NF-κB activity abrogates p53-dependent apoptosis. In addition, NF-κB activity is required for the anti-CD3-mediated apoptosis of DP thymocytes (Hettmann et al. 1999). NF-κB/Rel has also cell-death inducing activity in progenitor B cells, where in the presence of increased NF-κB activity cytokine-withdrawal promotes apoptosis, showing repression of Bcl-2 expression (Sohur et al. 1999; Sohur et al. 2000). So depending on the cellular context, NF-κB may...
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both promote or protect against programmed cell death.

IRF-1

IRF-1 is a transcription factor that activates type I interferon (IFN) and IFN-inducible genes, has tumor-suppressive activities and when inactivated may be linked to the development of hematopoietic malignancies (Tanaka et al. 1994; Tani-guchi et al. 1997). As discussed earlier, DNA damage-induced apoptosis in mitogen-activated mature T lymphocytes is regulated by an p53-independent pathway (Strasser et al. 1994), as opposed to p53-dependent apoptosis signaling in primary thymocytes (Lowe et al. 1993). Studies in IRF1-deficient mice have indicated that the former form of DNA damage-induced cell death in T lymphocytes depends on IRF-1, because activated splenocytes lacking IRF-1 are resistant to apoptotic cell death after treatment with γ-radiation or genotoxic drugs (Tamura et al. 1995). Furthermore, mitogen-dependent induction of ICE/Caspase 1 is dependent on IRF-1 (Tamura et al. 1995; Tamura et al. 1997).

Many transcription factors that are activated upon TCR engagement, and which are involved in the transcriptional activation of cytokine genes, have been implicated in the control of FasL expression. TCR-inducible FasL expression critically depends on the IRF-1 binding site in the FasL promoter, since mutation or deletion of this site results in deficient FasL expression. Additionally, suppression of IRF-1 expression in T cells results in deficient FasL expression (Chow et al. 2000). Therefore at least two different apoptosis-signaling cascades are regulated by IRF-1, including DNA damage-induced apoptosis as well as the Fas pathway.

Glucocorticoid receptor

The glucocorticoid receptor (GR) is a member of a large superfamily that includes receptors for other steroid hormones and a number of orphan receptors, like Nur77 (see below). At the carboxy-terminus of these receptors the ligand-binding domain resides that is also required for hormone-dependent gene transactivation, a central zinc-finger-containing DNA binding domain, and an N-terminal variable region important for ligand-independent gene transactivation (Beato et al. 1995). Normally the GR exists in the cytosol in complex with heat shock proteins, and it translocates to the nucleus when it is occupied by ligand. Within the nucleus, GR binds as a homodimer specific DNA sequences that make up the GRE (glucocorticoid responsive elements), where it enhances or inhibits transcription of the corresponding genes (Beato 1991).

GR-induced signaling can result in many biological activities, including immunosuppressive and anti-inflammatory effects. This relates to the fact that glucocorticoids are able to induce apoptosis in lymphoid cells. Supra-physiological levels of cortisone mediate especially depletion of B220+ slg+ precursor B cells (Garvy et al. 1993) as well as CD4+CD8+ DP thymocytes (Wyllie 1980). The fact that mainly CD4+CD8+ DP thymocytes are exclusively sensitive for corticosteroid-induced cell death has been related to expression levels of Bcl-2. Resting peripheral T cells and TCRhi CD4+ or CD8+ SP thymocytes, which are comparatively resistant to glucocorticoid-induced apoptosis (Cohen and Duke 1984), display reasonable expression of Bcl-2, which is not the case in CD4+CD8+ cells (Hockenbery et al. 1991). In addition, mature T cells derived from Bcl-2-deficient ES cells are equally sensitive as CD4+CD8+ thymocytes to apoptosis induced by glucocorticoids (Nakayama et al. 1993).

The mechanism(s) by which GR causes apoptosis is still largely unknown. Glucocorticoid-induced apoptosis is diminished by inhibitors of mitochondria-dependent cell death such as Bel-2 and Bcl-x; (Sentman et al. 1991; Siegel et al. 1992; Grillot et al. 1995) as well as IAPs (inhibitors of apoptosis) (Deveraux et al. 1997; Roy et al. 1997; Deveraux et al. 1998), and requires Apaf-1 (Yoshida et al. 1998) and caspase-9 (Hakem et al. 1998; Kuida et al. 1998). Since GR is a transcriptional regulator, it most likely controls the expression of one or more gene products that are important in mediating programmed cell death. This is supported by the finding that corticosteroid-induced apoptosis in thymocytes is prevented by inhibitors of protein synthesis (Thomas et al. 1983). Furthermore thymocytes, derived
from mice carrying a mutant GR unable to dimerize and therefore transactivate, are refractory to glucocorticoid-induced apoptosis (Reichardt et al. 1998; Tranche et al. 1998). Alternatively, GR may also modify gene transcription via direct protein-protein interactions with other transcription factors, such as AP-1 and NF-κB (Cato and Wade 1996).

Interestingly, there seems to be an additional positive role for the GR during T cell development. The physiological relevance for high GR expression levels in thymocytes is to respond to locally produced glucocorticoids by thymic epithelium (Vacchio et al. 1994) (Pazirandeh et al. 1999). The functional significance of these low glucocorticoid concentrations may be to antagonize TCR-mediated apoptosis and allow survival of thymocytes. This is based on results showing that stimulation through either TCR or GR alone induces apoptosis, whereas simultaneous signaling through both receptors paradoxically rescues thymocytes as well as T-cell hybridomas from cell death (Zacharchuk et al. 1990; Iseki et al. 1991; Iwata et al. 1991). It has been proposed that DP thymocytes with subthreshold avidity for antigen-self-MHC undergo death (by neglect) at least in part because of glucocorticoid-induced apoptosis. TCR-mediated signaling imposed by intermediate, but not high avidity interaction, with antigen-self-MHC in combination with glucocorticoids may induce antagonism, resulting in thymocyte survival (positive selection).

The functional implication of this mutual antagonism model is that decreasing glucocorticoid/GR levels or responsiveness should affect antigen-specific thymocyte selection by causing the activation-induced death of cells that would normally be positively selected. In other words, levels of TCR-mediated signaling that would under normal circumstances induce positive selection now result in negative selection. Indeed, pharmacological blocking of corticosteroid production in fetal thymic organ culture (FTOC) makes thymocytes more sensitive to cell death induced by anti-TCR antibodies or low avidity ligands (Vacchio et al. 1994; Vacchio and Ashwell 1997; Vacchio et al. 1999).

The exact role of GR-induced signaling for in vivo intrathymic T cell development has been investigated in two independent antisense-GR transgenic lines (King et al. 1995; Morale et al. 1995), a GR point-mutant “knock-in” (Reichardt et al. 1998), and recently in GR-null mutant mice (Purton et al. 2000). In one antisense transgenic strain T cell development is significantly disturbed, with 90% reduction of thymus size in homozygous transgenic mice, due to a decrease in the number of DP thymocytes and a secondary decrease in CD4+CD8" and CD4"CD8" thymocytes, whereas heterozygous mice have an intermediate phenotype (King et al. 1995). However, in the three other independent (partially) GR-deficient mouse models T cell development proceeded normally, despite significant resistance to glucocorticoid-induced apoptosis. Furthermore, in GR-null mutant mice negative selection, mediated by superantigen staphylococcal enterotoxin B (SEB), or anti-CD3/CD28, is also normal (Purton et al. 2000). Therefore, it is reasonable to conclude that GR signaling is not essential for intrathymic T cell development or selection.

Orphan steroid receptors Nur77/NOR1

The orphan steroid receptor Nur77/NGFI-B was originally identified as an immediate early gene transiently induced by serum, growth factors, and NGF (Hazel et al. 1988; Milbrandt 1988; Ryseck et al. 1989; Nakai et al. 1990). Together with Nurrl and NOR1 they constitute the NGFI-B subfamily (Maruyama et al. 1998). Heterodimers of the different members are more potent transcriptional activators than homodimers after binding to the Nur response element (NurRE) (Maira et al. 1999). However, unlike most steroid receptors that bind DNA as dimers, Nur77 can bind the NBRE site as a monomer (Wilson et al. 1991; Wilson et al. 1993). Several lines of evidence implicate induction of the Nur77 in activation-induced cell death in T-cell hybridomas and thymocytes. First, differential hybridization shows that the immediate-early gene NGFI-B (nur77) is induced in T cell hybridomas or in thymocytes undergoing apoptosis, indicating that Nur77 expression correlates with TCR-mediated apoptosis (Liu et al. 1994; Woronicz et al. 1994). Second, expression of Nur77 correlates with positive and negative thymic selection using TCR-transgenic mouse studies (Xue et al. 1997). Third, blocking
Nur77 mRNA expression with a dominant-negative construct in transgenic mice protects thymocytes against TCR-induced apoptosis (Calnan et al. 1995; Zhou et al. 1996), while overexpression of full-length Nur77 shows the reverse phenotype (Calnan et al. 1995; Weih et al. 1996).

The exact role of NGFI-B family members in slgM-induced apoptosis in B cells remains to be established. Until now it has been shown that Nur77 expression is induced after crosslinking slgM on resting B cells (Mittelstadt and DeFranco 1993). Studies in Burkitt lymphoma cell lines have indicated that induction of Nur77 expression strongly correlates with sensitivity to slgM-mediated apoptosis (Mapara et al. 1995), which could indicate a role for Nur-77 in slgM-mediated apoptosis of immature B cells.

Some data suggest that Nur77-induced apoptosis acts upstream of the Fas: FasL death pathway, since increased FasL expression levels are detected in Nur77-transgenic thymocytes and on a FasL-deficient gld background, thymus cellularity and thymocyte subpopulations are substantially restored to normal levels (Weih et al. 1996). However, in another Nur77-transgenic strain (Nur77-FL) as well as NOR1-FL transgenic mice, both cell surface and mRNA expression levels of FasL are identical with non-transgenic controls, although a similar apoptosis phenotype is observed in these mice (Calnan et al. 1995; Cheng et al. 1997). Since Nur77-FL transgene rescues the T cell specific aspects of the lymphoproliferative disease of gld/gld mice, it seems more likely that FasL is not a major downstream target of Nur77 (Chan et al. 1998). Constitutive expression of Nur77 in thymocytes does not enhance the sensitivity to glucocorticoid-induced apoptosis, and overexpression of Bcl-2 can not rescue apoptosis in Nur77-FL transgenic mice (Cheng et al. 1997).

Mice expressing a transcriptionally less active version of Nur77 display mild apoptosis, whereas overexpression of a more transcriptionally active version induces massive apoptosis (Kuang et al. 1999), indicating that Nur77 transcriptional activity correlates with its apoptotic function. However, Nur77-deficient thymocytes show normal anti-CD3-mediated T cell death and development (Lee et al. 1995). This has been attributed to expression of the redundant and functional homologue Nor1 (Cheng et al. 1997). Functional analysis in Nor1-single and Nor1/Nur77-double deficient mice needs to reveal the definitive role of these orphan steroid receptors in T cell development and negative selection.

MEF2

The MEF2 family of transcription factors comprise of MADS-box proteins, which are involved in diverse cellular processes, including muscle and neuronal differentiation (Gossett et al. 1989; Pollock and Treisman 1991; Yu et al. 1992; Leifer et al. 1993; Martin et al. 1994; Black and Olson 1998). However, various data implicate MEF2 also as an important regulator of T cell apoptosis, through its ability to regulate Nur77 expression. The calcium-signaling pathway is important in controlling Nur77 induction, whereas protein kinase C signals only induce a low level of Nur77 activity (Woronicz et al. 1995). Two calcium-responsive DNA elements are present in the Nur77 promoter, which form the consensus binding sites for the MEF2 transcription factor. These observations implicate MEF2 as a Ca$^{2+}$-dependent transcription factor for Nur77 expression.

Cabin 1 is an endogenous inhibitor of the protein phosphatase calcineurin, and sequesters MEF2 in a transcriptionally inactive. TCR signaling leads to a rise in intracellular Ca$^{2+}$ concentrations and dissociation of MEF2 from Cabin 1, by competition of calmodulin binding to Cabin1. Repression of MEF2 by Cabin1 involves recruitment of mSin3 and its associated histone deacetylases and competition with co-activator p300/CBP for binding to MEF2 (Youn and Liu 2000). Recruitment of co-activator p300 to MEF2 is enhanced by NFAT (Youn et al. 2000). Thus MEF2 may integrate different Ca$^{2+}$ signaling pathways regulating Nur77 expression (Blaeser et al. 2000).

Orphan retinoic acid receptor RORγ
The retinoic acid receptor-related orphan receptors RORα, RORβ and RORγ constitute a subfamily of nuclear orphan receptors (Becker-Andre et al. 1993; Carlberg et al. 1994; Giguere et al. 1994; Hirose et al. 1994). Each of these receptors binds as a monomer to a specific response elements (ROREs) (Giguere et al. 1995; Medvedev et al. 1996). RORγ is expressed in thymus, kidney, liver, skeletal muscle, but not in mature peripheral T cells (Ortiz et al. 1995). Overexpression of RORγ inhibits FasL and cytokine gene expression and protects hybridomas from TCR-induced apoptosis (He et al. 1998). RORγ-deficient mice show reduced number of thymocytes and increased sensitivity to apoptosis due to loss of Bcl-xL expression levels (Kurebayashi et al. 2000; Sun et al. 2000). It is not clear yet whether this involves direct or indirect regulation of bcl-xL expression by RORγ. RORγ-deficiency phenocopies in this respect the defect of bcl-xL−null thymocytes (Ma et al. 1995). Transgene-driven expression of Bcl-xL restores most aspects of normal thymocyte development in RORγ−/− mice (Sun et al. 2000).

Crosses between TCRα−/− and RORγ−/− indicate that in the absence of TCR expression on the cell surface RORγ−/− thymocytes still display the same apoptosis phenotype, indicating that negative selection signals do not initiate premature apoptosis in the absence of RORγ (Sun et al. 2000). Crossing RORγ−/− animals with FasL-deficient gld/gld mice shows that also the Fas:FasL system is not regulated by RORγ, since thymocyte apoptosis in gld/gldRORγ−/− mice is identical to RORγ−/− mice (Sun et al. 2000). Therefore, regulation of FasL expression by RORγ seems not to be critical for inducing apoptosis.

Scope of this thesis

Deregulation of critical signaling pathways implicated in controlling apoptosis in lymphoid cells has a major impact on T and B cell physiology. In case potentially auto-reactive T cells are not eliminated from the thymus, T cell stimulation and subsequent activation could occur after encountering specific peptide-self MHC molecules, which may result in autoimmune disease. Alternatively, sustained lymphoid cell survival may impose a certain risk in the development of cancer. Data presented in this thesis provide strong indications that improper transcriptional control of apoptosis holds a major risk in the development of lymphoid tumors. The Polycomb group protein Bmi1 and the SNAG-domain transcriptional repressor Gfi1 represent two new classes of transcriptional regulators important for apoptosis control in lymphoid cells. Furthermore, their ability to control distinct Myc-mediated apoptosis signaling pathways, procure important clues how Bmi1 and Gfi1 collaborate with the c-myc proto-oncogene in lymphomagenesis.

Polycomb group protein Bmi1

The mouse bmi1 gene was identified as a common proviral integration site of Moloney murine leukemia virus (MoMLV) in Eμ-myc (pre-) B cell lymphomas (van Lohuizen et al. 1991b). Bmi1 and its close homologue Mel18 contain a conserved RING finger motif (Tagawa et al. 1990; Ishida et al. 1993) and represent the mammalian orthologues of the Drosophila Polycomb genes posterior sex combs and suppressor 2 of zeste (Brunk et al. 1991; van Lohuizen et al. 1991a).

Murine Polycomb group (Pc-G) proteins engage in two distinct multimeric complexes: one complex includes Eed, Enxl/EzH2, and Enx2/EzH1 (Denisenko et al. 1998; Sewalt et al. 1998; van Lohuizen et al. 1998) and the other Bmi1, Mel18, Mphl/Rae28, and M33 (Alkema et al. 1997; Gunster et al. 1997; Satijn et al. 1997; Satijn and Otte 1999). Pc-G protein-complexes bind to Polycomb Response Elements (PRE) (Simon et al. 1993; Chan et al. 1994; Chiang et al. 1995), which are usually a few kilobases in size, and induce a change in the chromatin configuration that becomes inaccessible to transcriptional activators (Pirrotta 1997; Pirrotta 1998). Although some individual Pc-G members can bind DNA sequence-specific and display transcription repression activity (Kanno et al. 1995; Brown et al. 1998), the assembly of a complete Pc-G complex is necessary for the formation of a stable silencing complex at a PRE.

Pc-G proteins propagate stable transcriptional repression of homeotic genes, which is re-
required for correct diversification of the body plan during embryonic development. Loss of function mutations of bmil (van der Lugt et al. 1994), mell8 (Akasaka et al. 1996), and M33 (Core et al. 1997) or ectopic transgenic expression of bmil (Alkema et al. 1995) result in dosage-sensitive homeotic transformation of the axial skeleton. This is accompanied by shifts in specific Hox-gene expression boundaries. In addition, retarded growth, neurological and hematopoietic defects are observed in several of the Pc-G mutant animals, including bmil'' mice. In the absence of Bmil, these proliferative defects result from increased transcriptional activity of the INK4A/ARF locus, since introduction of the INK4A/ARF-null mutation in Bmil-deficient mice rescues to a large extent the cellular defects in cerebellum, spleen and thymus (Jacobs et al. 1999).

However, correction of aberrant INK4A/ARF expression in bmil'' mice is also essential to rescue the increased apoptosis levels observed in lymphocytes of Bmil-mutant mice (chapter 4). Furthermore, the reduced cellularity and increased levels of programmed cell death in bmil'' mice are also partially rescued by overexpression of Bcl-2 (chapter 4). Regulation of INK4A-ARF expression by Bmil is essential to inhibit Myc-induced apoptosis, and allow for full oncogenic transformation by c-Myc (chapter 4).

SNAG-domain transcriptional repressor Gfi1

The gfi1 gene was cloned by its virtue to confer IL-2-independent cell growth to a T cell lymphoma cell line (Gilks et al. 1993). Subsequently, gfi1 was implicated as a potential proto-oncogene by the finding that proviral transcriptional activation of gfi1 expression is a frequent event in MoMLV-induced T cell and to a lesser extent in B cell tumors of c-Myc and pim transgenic mice (chapter 2; Zörnig et al. 1996)). Gfi1 contains six zinc-finger motifs in the carboxy-terminal half of the protein, which harbor the specific DNA-binding domain (Zweidler-Mckay et al. 1996). At the extreme N-terminus the autonomous transcriptional repression domain resides, which is also present in the Snail/Slug family of transcription factors, and has been termed SNAG-domain (Grimes et al. 1996). The Gfi1 homologue Gfi1B shows high sequence homology in the zinc-finger domain and transcription repression domain (Tong et al. 1998). Whereas both gfi1 and gfi1B are highly expressed in bone marrow, gfi1 is more strictly expressed in thymus and gfi1B in spleen (Tong et al. 1998).

Gfi1 promotes cell cycle entry in the absence of IL-2 (Grimes et al. 1996a; Zörnig et al. 1996), and cell survival by repressing expression of bax and bak in primary T cells (Grimes et al. 1996b). However, Gfi1 also fulfills other anti-apoptotic functions independent of Bcl-2 expression levels (chapter 5 and 8). One line of evidence, relates to the fact that Gfi-1 inhibits different modes of T cell receptor and death-receptor-mediated apoptosis without providing protection against DNA damage-induced cell death or absence of survival factors (chapter 5). These last two forms of induced cell death are normally most effectively inhibited by Bcl-2 or Bcl-xL action. Secondly, activation of Myc in the context of Gfi1 overexpression efficiently transforms thymocytes and provides synergistic protection against glucocorticoid- and PMA-induced apoptosis, in the presence of severely reduced Bcl-2 and Bcl-xL levels (chapter 8). In addition, there seems no selection pressure to mutate the p53 pathway in Gfi1/c-Myc transformed.

Recent data indicate that Gfi1 enhances STAT3-mediated signaling and augments IL-6-dependent cell proliferation (Rödel et al. 2000). In addition, gfi1 overexpression alters early pre-T and subsequent immature T cell differentiation (chapter 5; Schmidt et al. 1998b). Like Gfi1B (Tong et al. 1998), Gfi1 is also able to control myeloid cell differentiation, proliferation and apoptosis, which is illustrated by the finding that gfi1 transgenic mice show a clear increased risk of developing chronic myeloid leukemia (CML)(chapter 6). Furthermore, overexpression of Gfi1 predisposes to the onset of lymphoblastic T cell lymphomas (chapter 6; Schmidt et al. 1998a). These data illustrate that Gfi1 is an important regulator of thymocyte apoptosis signaling.

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