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Regulation of apoptosis in transgenic mice by simian virus 40 T antigen-mediated inactivation of p53

(thymocytes/irradiation/clonal deletion/tumor suppressors/tumorigenesis)

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ABSTRACT **Several proteins encoded by DNA tumor viruses are thought to disrupt cellular growth control by interacting with key cellular proteins, such as p53 and pRB, that normally function to regulate cell growth. However, the biological consequences of intracellular complexing between the viral oncoproteins and cellular proteins have remained unclear. Such complexes could either facilitate functional inactivation of the cellular proteins, leading to a loss-of-function phenotype, or could activate new functions, leading to a gain-of-function phenotype. Here we demonstrate that the simian virus 40 large tumor (T) antigen produces a loss-of-p53-function phenotype when introduced into the thymocytes of transgenic mice. Like thymocytes from the recently characterized p53-null mice, thymocytes from transgenic mice expressing a T-antigen variant capable of binding to p53 are resistant to irradiation-induced apoptosis. Thymocytes from transgenic mice expressing a mutant T antigen that is unable to complex p53, but retains the ability to complex the pRB and p107 proteins, retain sensitivity to irradiation. We further demonstrate that although irradiation-induced apoptosis is impaired by T antigen, clonal deletion of autoreactive thymocytes via p53-independent apoptosis is not perturbed by T antigen. These results provide convincing evidence that T antigen inactivates p53 in thymocytes *in vivo* and suggest a mechanism by which T antigen predisposes thymocytes to tumorigenesis in T antigen-transgenic mice.**

The transforming proteins from at least three distinct DNA tumor viruses, adenovirus, simian virus 40 (SV40), and oncogenic human papillomaviruses (HPVs), interact with a common subset of cellular proteins to disrupt normal cellular growth regulation. At least two of these cellular proteins, pRB and p53, may function to negatively regulate cell growth and are often mutated in human tumors (see refs. 1–3 for review). These observations have led to the hypothesis that the ability of the DNA tumor virus proteins to stimulate cell growth reflects the release of a brake on cell proliferation normally imposed by proteins such as p53 and pRB (4). However, the specific biological consequences of each viral/cellular protein interaction are poorly understood. Although inactivation of negative regulators is one possible route to the induction of tumorigenic changes, another possibility is that these protein interactions induce new dominant activities. This possibility is supported by the observation that certain mutant forms of p53 appear to have dominant oncogenic activities that are not obviously the result of transdominant inhibition of normal p53 function (1).

The consequences of viral protein interaction with p53 are perhaps best understood in the case of the HPV-16 E6 protein. The interaction between E6 and p53 results in rapid degradation of p53 via the ubiquitin pathway (5). Hence, E6 appears to indeed function ultimately to inactivate p53. In contrast, however, the interaction of p53 with SV40 T antigen and with the adenovirus E1B 55-kDa protein results in stabilization of p53 (6, 7). Whether stable complex formation and degradation represent two different mechanisms that serve ultimately to inactivate p53 is not known. SV40 T antigen, HPV-16 E6, and adenovirus E1B have been shown to inhibit p53-mediated transcriptional regulation in cotransfection assays *in vitro* (8–11), and specific DNA binding by p53 is also inhibited by T antigen *in vitro* (12). Recently, HPV-16 E6 expression in tumor-derived cultured epithelial cells was shown to induce a p53-null phenotype, in that the cells were no longer able to arrest in G_1 following DNA damage (13). Although these *in vitro* experiments suggest that p53 function is inactivated by interaction with viral proteins, no *in vivo* biological test for the effect of viral proteins on p53 function under physiological conditions has been carried out. Understanding the consequences of these protein interactions will be key to elucidating the mechanism by which the viral proteins and their targeted cellular pathways function.

We have approached this problem by analysis of transgenic mice carrying both wild-type and mutant forms of the SV40 large-T-antigen gene under the control of a tissue-specific promoter (14–17). We have so far compared several transforming functions of T antigen in a brain epithelial cell type (the choroid plexus) and in lymphoid cells. Analyses of tumor induction by several mutant T antigens suggest that the ability of T antigen to induce tumorigenesis in T lymphocytes correlates with its ability to bind p53 (15, 16). In the present work, we have continued to explore the mechanism by which p53-interacting forms of T antigen induce T-cell tumors. We have done so by testing and confirming the hypothesis that T antigen creates a functional p53-null state in thymocytes, leading to inhibition of a critical apoptosis pathway that might normally eliminate tumorigenic cells.

MATERIALS AND METHODS

Mouse Lines. The transgenic mouse lines LST1135-11 (16) and LST1137-5 (17) harbor variant T-antigen genes (Fig. 1) and were generated in previous studies according to standard pronuclear injection techniques (15) followed by breeding of the founder BDF_2 transgenic mice to the hybrid BDF_1 background (the F_1 progeny of a cross between DBA/2 and C57BL/6J inbred mice). Hence, each transgenic mouse is on a mixed DBA/2J and C57BL/6J background. BDF_1 mice were also used for normal control mice and were obtained from The Jackson Laboratory. Mice deficient in the p53 locus (generously provided by Tyler Jacks, Massachusetts Institute of Technology Cancer Center), originally on a hybrid (C57BL/6 × 129/sv) background, were bred as heterozy-

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Abbreviations: HPV, human papillomavirus; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; SV40, simian virus 40.

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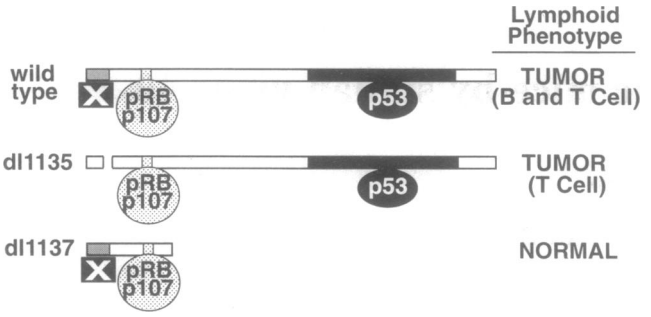

FIG. 1. Diagrams of the wild-type and the dl1135 and dl1137 mutant SV40 T-antigen proteins. Established transformation-related activities of the proteins are represented by shaded regions, from N-terminus to C-terminus, as follows: the genetically defined N-terminal transforming activity, indicated with an X, is absent from the dl1135 protein: the pRB/p107-binding region involves amino acids 105–114 in all three proteins; and the p53-binding region, which maps to amino acids 271–517, is present in the wild-type and dl1135 proteins but absent from dl1137. The phenotypes of each protein with respect to B- and T-cell tumorigenesis are indicated. Founder mice expressing wild-type T antigen under control of the promoter used in this study rarely develop B- and T-cell lymphoma, due to early death from brain tumors (14, 15). Wild-type T antigen under other B-cell- and T-cell-specific promoters routinely develop B- and T-cell lymphoma (38, 39).

gotes to BDF_1 mice for several generations before breeding to homozygosity at the mutant p53 locus for use as controls in the present experiments. All mice were screened for the appropriate genomic alteration by PCR analyses of tail DNA (16–18).

Thymocyte Apoptosis Assays. Thymocytes from 3- to 20-week-old BDF_1 control mice, a 4-week-old p53-null mouse, and 3- to 8-week-old LST1135 mice and LST1137 mice (Fig. 1) were assessed for induction of DNA fragmentation (19, 20). In brief, irradiated or unirradiated cells were cultured overnight with one or more stimuli, as indicated. After culture, the cells were washed, lysed, and microcentrifuged to separate intact DNA (in the pellet) from fragmented DNA (in the supernatant). The pellets and supernatants were adjusted to equal volume and plated in serial dilutions. The fluorescent DNA-binding reagent 4′,6-diamidino-2-phenylindole (DAPI) was added to the wells and the fluorescence was quantitated on a plate reader. The titration curves were mathematically converted to percent DNA fragmentation = 100 × [DNA in supernatant/(DNA in supernatant + DNA in pellet)]. We confirmed that the DNA in fragmented samples showed the characteristic DNA ladder pattern by gel electrophoresis (see *Results*). In Fig. 2, dexamethasone was used at 1 μM, A23187 was used at 250 ng/ml, phorbol 12-myristate 13-acetate (PMA) was used at 10 ng/ml, and irradiation of cells before culture was 485 rads from a Gammacell cesium-137 source at 1615 rads/min (1 rad = 0.01 Gy). The p53-null thymocytes received 808 rads. In additional experiments not shown, dexamethasone was used over a wide concentration range (0.1–4.0 μM); control and transgenic thymocytes exhibited equivalent DNA fragmentation. Irradiation was also tested over a wide dose range (162–2100 rads); control thymocytes were sensitive over the full range, whereas LST1135 thymocytes were resistant over the full range (data not shown). It should be noted that the dose response for A23187 plus PMA is quite complex and that other doses (i.e., A23187 at 100–125 ng/ml plus PMA at 10 ng/ml) induce a protective mechanism that inhibits apoptosis in normal control thymocytes (19, 20).

DNA-Ladder Assay. After overnight exposure to the apoptotic stimuli listed above (see *Thymocyte Apoptosis Assays*), thymocytes were quick-frozen in liquid nitrogen for later analysis of DNA ladder formation. The frozen cells were thawed quickly at room temperature in 1 ml of extraction buffer (10 mM Tris Cl, pH 8.0/0.1 M EDTA, pH 8.0/0.5% SDS with pancreatic RNase A at 20 μg/ml and proteinase K at 100 μg/ml). Once suspended, the samples were incubated at 55°C for 3 hr and then cooled to room temperature. The highly viscous DNA solutions were carefully extracted twice with an equal volume of buffer-saturated phenol (pH 8.0) by using a wide-bore pipet. After ethanol precipitation, the samples were suspended in 300 μl of TE (pH 8.0) containing RNase A at a final concentration of 8.0 μg/ml. After electrophoresis in a 1.5% agarose gel, the DNA ladders were visualized by staining the gel with ethidium bromide at 0.5 mg/ml.

Analyses of T-Cell Antigen Receptor (TCR) β-Chain Variable-Region (V_β) Subsets. Spleen cells from 3- to 20-week-old C57BL6/J, BDF_1, LST1135, and LST1137 mice were depleted of erythrocytes, and 0.5–1.0 × 10^6 cells were aliquoted for staining. Prior to staining, unlabeled 2.4G2 anti-Fc receptor monoclonal antibody (mAb) was added to the cells to prevent nonspecific binding of labeled mAbs to Fc receptor-positive cells. Staining was performed as described (16). Negative control staining was done with biotinylated anti-human CD3 mAb (anti-Leu-4; Becton Dickinson). For each mouse, the percent positive cells in its anti-Leu-4 stain was subtracted from the percent positive cells in each TCR stain to determine the percent V_β-positive or αβ TCR-positive cells. For each TCR V_β stain in each mouse, percent of T cells = 100 × (percent V_β-positive/percent αβ-positive).

RESULTS

Characteristics of Transgenic Mouse Lines. To investigate whether binding of p53 by SV40 large T antigen functionally inactivates p53 *in vivo*, we produced transgenic mice carrying variants of T antigen that do (dl1135) or do not (dl1137) form intracellular complexes with p53 (Fig. 1). T-antigen expression was directed to thymocytes as well as mature T lymphocytes, B lymphocytes, and brain choroid plexus epithelium by using the transcriptional regulatory signals of the lymphotropic papovavirus (14). Production of the transgenic mice and characterization of tumor development were described previously (16, 17). Expression of dl1135 in transgenic mice results in the consistent development of thymic lymphoma (16), whereas dl1137 fails to induce obvious thymic abnormalities (15, 17). The inability of dl1137 to induce thymic lymphoma is not a result of inactivity of the protein, since dl1137 is sufficient to induce tumors in brain choroid plexus epithelium (15, 17). Transgenic mice expressing wild-type T antigen under the same promoter were not used in this study, because the majority of founder animals die of brain tumors prior to breeding age, precluding the assessment of lymphoma in established lines (14). In dl1135-expressing lymphomic tissue, all of the detectable p53 is complexed to approximately half of the available dl1135 protein (16). In contrast, the dl1137 T antigen does not contain the p53-binding domain and does not form a complex with p53 (15, 21). To elucidate the mechanism for the differential ability to induce thymic tumors by these proteins, we analyzed the apoptotic pathways in thymocytes from representative families of mice that express either dl1135 or dl1137 (referred to as LST1135 and LST1137 mice, respectively).

T Antigen Induces a p53-Null State in Transgenic Thymocytes. The LST1135 mice develop clonal tumors despite the fact that T antigen is expressed in all thymocytes prior to the development of tumors (16). We therefore postulated that T-antigen expression predisposes thymocytes to tumorigenesis by inactivating a p53-dependent cellular surveillance system that would normally eliminate tumorigenic cell clones. To test this hypothesis, we analyzed LST1135 thymocytes prior to the time when detectable lymphomic cells appear. We initially focused on the response of thymocytes to irradiation, which may mimic *in vivo* mutagenic events.

Irradiation induces apoptosis in thymocytes by an intracellular activation process that has recently been shown to require functional p53 (18, 22). We irradiated single-cell suspensions of fresh murine thymocytes, cultured the cells overnight, and quantitated apoptosis. In parallel, we cultured thymocytes with stimuli that induce p53-independent apoptosis, to investigate whether any intracellular effects of T-antigen expression were specific for p53-associated processes. Apoptosis was monitored by quantitation of cellular DNA fragmentation, which has been shown to directly correlate with apoptotic death (23, 24).

Fig. 2*A* illustrates the results of a large number of experiments comparing control BDF_1, p53-null, LST1135, and LST1137 thymocytes. Thymocytes from all mouse strains tested, including the LST1135 transgenic mice, were readily induced to undergo apoptosis *in vitro* in response to p53-independent stimuli such as dexamethasone and the combination of the Ca^{2+} ionophore A23187 and the phorbol ester PMA (Fig. 2*A*). This result demonstrates that T antigen does not globally disrupt the apoptotic machinery in LST1135 thymocytes. In contrast to the normal apoptotic response of LST1135 thymocytes to p53-independent stimuli, these thymocytes were unresponsive to the p53-dependent irradiation stimulus. Like thymocytes from p53-null mice, thymocytes from transgenic mice expressing dl1135 T antigen (p53-binding) were resistant to radiation-induced apoptosis (Fig. 2*A*). In contrast to p53-null mice and LST1135 transgenic mice, LST1137 (p53-nonbinding) transgenic mice and nontransgenic BDF_1 mice exhibited normal thymocyte sensitivity to radiation-induced apoptosis (Fig. 2*A*). Costimulation with irradiation plus dexamethasone, or irradiation plus A23187 and PMA, induced apoptosis in LST1135 thymocytes as well as in control thymocytes (Fig. 2*A*), demonstrating that T-antigen expression does not nonspecifically preclude activation of an apoptotic response in irradiated thymocytes. As expected, the DNA from thymocytes undergoing apoptosis was fragmented and exhibited the characteristic "ladder" pattern (Fig. 2*B*); furthermore, the extent of DNA fragmentation correlates well with the results in Fig. 2*A*. These results strongly support the hypothesis that intracellular p53 is inactivated by T antigen in the LST1135 cells, creating a p53-"null" loss-of-function phenotype.

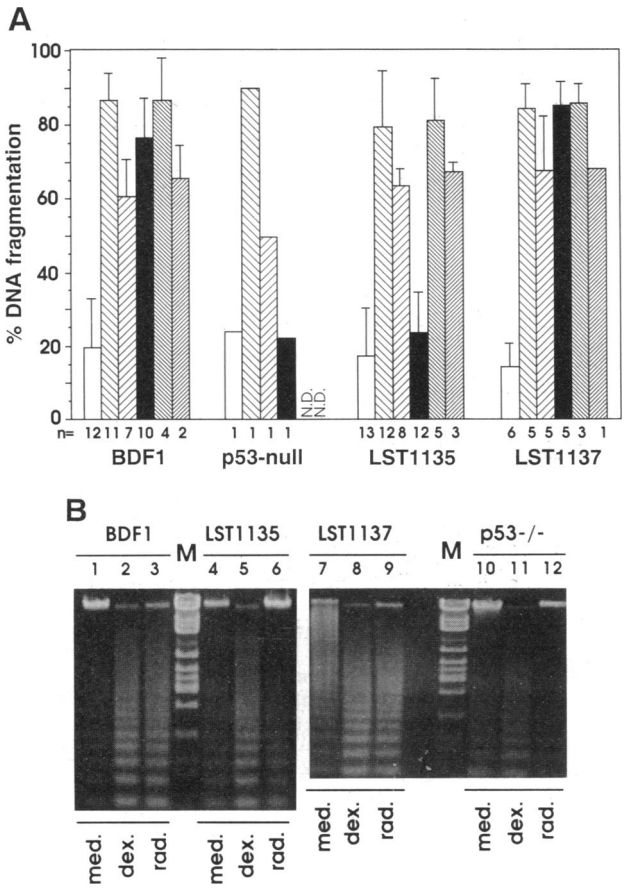

FIG. 2. (*A*) Effect of SV40 T-antigen expression on thymocyte responses to apoptotic stimuli. Single-cell suspensions of thymocytes were cultured for 20–24 hr with the indicated stimuli. Percent DNA fragmentation in the cultured cells was assessed in the quantitative assay described in *Materials and Methods* and corresponds to percent apoptosis. The mouse genotypes from which the thymocytes were obtained are indicated along the *x* axis. *n*, Number of individually tested mice for each stimulation condition. □, Medium; ▨, dexamethasone; ▨, A23 plus PMA; ■, γ-irradiation; ▨, γ-irradiation plus dexamethasone; ▨, γ-irradiation plus A23187 plus PMA; ND, not done. (*B*) DNA fragmentation resulting from apoptotic death. Thymocytes from 1-month-old BDF_1, p53−/−, LST1135, and LST1137 mice were isolated and incubated as single-cell suspensions in medium (med.), with dexamethasone (dex.), or under γ-irradiation (rad). Genomic DNA was isolated from the treated thymocytes, and ladders were visualized as described in *Materials and Methods*. M, size markers.

T Antigen Does Not Interfere with Clonal Deletion of Thymocytes in Transgenic Mice. Although LST1135 mice are extremely susceptible to thymic tumor development, they do not display other thymic abnormalities (16). For example, prior to overgrowth of the thymus with a clonal tumor the thymocyte subset distribution is normal, suggesting that a normal developmental progression is operating (16). Further, there is no evidence of T-lymphocyte autoimmunity in these mice, suggesting that normal intrathymic self-tolerance is also operating. In normal mice, the thymocyte developmental program includes the clonal deletion via apoptosis of autoreactive T cells prior to their final maturation (reviewed in ref. 25). Apoptosis is triggered in autoreactive thymocytes by TCR/CD3 binding to self-antigens, which initiates an intracellular signaling cascade that is partially mimicked in response to A23187 and PMA *in vitro* (19, 20, 25). LST1135 and p53-null thymocytes did respond to this stimulus (Fig. 2*A* and refs. 18 and 22), suggesting that p53 function, while required for irradiation-induced apoptosis, is not required for normal T-cell self-tolerance induction *in vivo*. We tested this prediction by analyzing the TCR repertoire of mature splenic T cells in LST1135 transgenic mice.

The LST1135 and LST1137 mice were produced on a BDF_1 background and are currently in the seventh generation of repeated crossing with BDF_1 for propagation of the line. The DBA/2 strain expresses independently segregating self-antigens that induce clonal deletion via apoptosis of developing thymocytes that express certain TCR, including those containing $V_\beta 3$, $V_\beta 6$, and $V_\beta 9$ (26). As a result, mature splenic T-cell populations from normal BDF_1 mice are deficient in cells expressing these TCR V_β regions (Fig. 3). To determine whether LST1135 mice were also able to delete this same subset of T cells, we examined the repertoire of splenic TCR V_β subtypes by flow cytofluorimetry. Mature T-cell populations from the LST1135 T-antigen-transgenic mice were routinely deficient in the DBA/2-deleted TCR subsets (Fig. 3), demonstrating that LST1135 mice undergo normal self-tolerance induction. The occasional TCR V_β nondeletion by an LST1135 mouse (one $V_\beta 3$ and one $V_\beta 9$) most likely reflects the fact that these individual transgenic mice did not inherit the particular DBA/2-derived self-antigens responsible for inducing those deletions, due to normal allele segregation during the heterozygous matings. In support of this interpretation, occasional nondeletion of TCR V_β subtypes was also observed in LST1137 mice (Fig. 3) and in nontransgenic littermates of the LST1135 mice (data not shown). If p53 binding had altered clonal deletion pathway(s) in LST1135

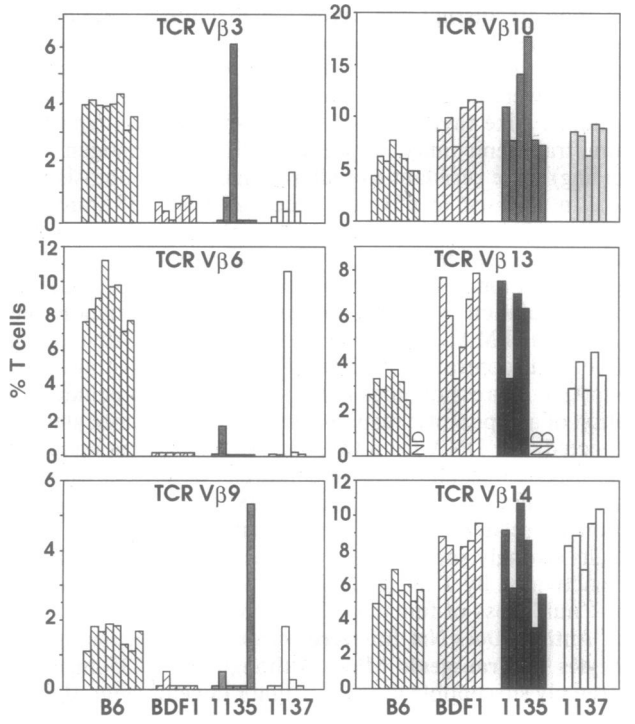

FIG. 3. TCR V_β-region usage by LST1135 transgenic mice. Single-cell suspensions of spleen cells from transgenic (LST1135 or LST1137) or nontransgenic (C57BL/6 or BDF_1) control mice were labeled with mAbs specific for the indicated TCR V_β regions and with a mAb specific for all TCR $\alpha\beta$-positive T cells. TCR V_β frequencies are expressed relative to the total frequency of $\alpha\beta$-positive T cells in each spleen. Eight C57BL/6 (B6) mice, six BDF_1 mice, six LST1135 (1135) mice, and five LST1137 (1137) mice were tested individually; each bar represents T cells from one mouse. C57BL/6 mice are nondeletors for all T-cell subtypes examined, whereas BDF_1 mice delete $V_\beta 3$, $V_\beta 6$, and $V_\beta 9$. Values ≤ 0.1 were plotted as 0.1 to make them visible.

mice, global rather than sporadic nondeletion of "forbidden" TCR V_β subtypes would have been expected in these mice. Together, the experiments shown in Figs. 2 and 3 thus demonstrate that functional p53, which is required for elimination of radiation-damaged immature T cells, is not required for elimination of autoreactive immature T cells *in vivo*. These results again emphasize the specificity of the intracellular effects of T-antigen expression and demonstrate that the inhibitory activity of T antigen on the apoptotic mechanism is mediated through p53.

DISCUSSION

The results demonstrate that SV40 large T antigen can functionally inactivate p53 *in vivo*. The inactivation of p53, either by T antigen (as described here) or by germline mutation (18, 22), results in increased susceptibility to thymic tumor development, perhaps due to the loss of a mutation-surveillance function of p53. Recently, p53 has been implicated as a checkpoint control in the G_1 phase of the cell cycle, due to its ability to arrest cells in G_1 and the observations that cells lacking p53 display genomic instability (27, 28) and fail to arrest in G_1 in response to DNA damage (29–33). The observation that p53 can induce apoptosis in certain cell lines also suggests that one possible consequence of p53-mediated G_1 arrest is cell death (34, 35). Such a function would be consistent with the observation that loss or mutation of p53 is associated with tumor development (1–3, 36).

Both p53-null mice (18, 22, 36) and LST1135 T antigen-transgenic mice (18) develop thymic lymphoid tumors with high frequencies; in LST1135 mice, this phenotype occurs with 100% penetrance. Our previous studies demonstrated that the thymomas in LST1135 transgenic mice consist of a mature population of T cells, usually $CD4^+$ $CD8^-$ TCR/$CD3^{hi}$ or $CD4^-$ $CD8^+$ TCR/$CD3^{hi}$ (16). Moreover, these tumors are clonal, suggesting that in addition to T-antigen expression, somatic mutations are required for tumor induction (14). Hence, T antigen-mediated inactivation of p53 could contribute to tumorigenesis by increasing the probability that cells with oncogenic mutations, which would normally be triggered to undergo apoptosis, could survive and proliferate. One possible source of oncogenic mutations in developing immature thymocytes is the process of TCR gene rearrangement. In normal mice, cells rendered defective or oncogenic by aberrant rearrangements may be eliminated by a p53-dependent apoptotic process, which is disrupted by T antigen in LST1135 mice (Fig. 4).

The model shown in Fig. 4 illustrates that p53 function is essential for apoptotic cell death during mutation surveillance in thymocytes. The model also incorporates the fact, originally shown with p53-null mice (18, 22), that not all thymocyte apoptosis requires p53 function. Here we show that LST1135 transgenic thymocytes also exhibit p53-dependent and p53-independent apoptotic pathways, and further demonstrate that apoptotic cell death during thymocyte self-tolerance induction (clonal deletion) *in vivo* proceeds normally in LST1135 mice without functional p53. Thus, the present studies demonstrate that at least two signaling pathways, with distinct physiological roles, can induce apoptosis in thymocytes. One pathway can be induced by radiation, is dependent on p53 function, and is inactivated by T antigen.

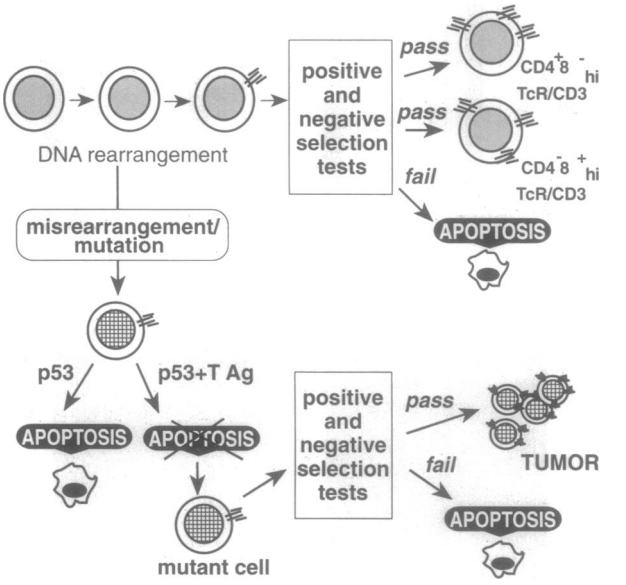

FIG. 4. Model for T antigen-induced predisposition to T-cell lymphoma. During early stages of differentiation, TCR genes rearrange, and the cells undergo changes in the expression of certain cell surface markers; for simplification only the CD4, CD8, CD3, and TCR markers of the mature T-cell populations are denoted here. During normal T-cell differentiation, cells expressing TCRs that fail to bind any ligand or that recognize self-antigens with high avidity are deleted via programmed cell death (apoptosis); *in vitro* treatment with A23187 and PMA may mimic these *in vivo* TCR-mediated selection processes. SV40 T antigen appears not to interfere with this apoptotic pathway (see text). In contrast, SV40 T antigen does interfere with irradiation-induced apoptosis in thymocytes, a pathway for which p53 function is necessary. In this model, cells that have undergone DNA damage as a result of TCR gene misrearrangement are proposed to require p53 for the induction of apoptosis. SV40 T antigen (Ag) interferes with this pathway, leading to the survival of cells that have accumulated mutations, some of which are oncogenic and lead to the outgrowth of a tumor. This model is consistent with results obtained in studies of T antigen-transgenic mice and p53-null mice (see text).

A second pathway or class of pathways is induced by A23187 and PMA (which together mimic TCR engagement) and/or by dexamethasone, is not dependent on p53 function, and is not inactivated by T antigen. The pathways may converge within the cell, since at least two genes, RP-2 and RP-8, are induced upon stimulation of apoptosis in thymocytes by either radiation or dexamethasone (37).

Although p53 function appears to be critical for mutation surveillance, other T antigen-targeted cellular proteins may also contribute to tumor suppression in thymocytes. This possibility is suggested by the fact that the penetrance of T-cell lymphomas (thymomas) in families of LST1135 mice is 100% (16), whereas the penetrance of lymphomas in p53-null mice is not complete (50–75%; refs. 1, 2, and 36). The pRB/p107-binding function of the T antigen in LST1135 mice may be responsible for this difference. By analogy with T antigen-mediated inactivation of p53 function, perhaps T-antigen inactivation of the function of one or more pRB-family members leads to an increased probability of tumorigenesis. The consequences of pRB/p107 functional inactivation in T cells are not known. However, pRB and p107 appear not to have critical functions in any of the known apoptotic pathways in thymocytes, since all apoptotic pathways tested were intact in LST1137 mice (in which the dl1137 T-antigen mutant binds pRB and p107 but not p53; Figs. 1 and 2). The pRB family of proteins may, therefore, regulate other processes, such as cell cycle progression (3).

In contrast to mice transgenic for the wild-type SV40 T antigen, which can develop both T-cell and B-cell tumors (14, 38, 39), LST1135 mice do not develop tumors of the B-lymphocyte lineage (16). The dl1135 T antigen variant has a small deletion near its N terminus (Fig. 1) which inactivates a T-antigen transformation domain (16, 21). The deletion in dl1135 T antigen may spare the functional activity of a cellular protein in LST1135 mice that is responsible for mutation surveillance in B cells. According to this scenario, both wild-type T antigen- and dl1137-transgenic mice would be deficient in this putative cellular protein activity in B cells. However, since only wild-type T-antigen mice and not LST1137 mice develop B-cell tumors, it is necessary to postulate that the functional activity of either p53 (in LST1137 mice) or the N-terminal-binding cellular protein (in LST1135 mice) is sufficient for mutation surveillance in B cells. In B cells expressing wild-type T antigen, both of these cellular activities would be inactivated (Fig. 1), leading to the induction of B-cell neoplasia. This hypothesis, although clearly speculative, provides a structure for future studies on B-cell apoptosis and tumor development in SV40 T antigen-transgenic and p53-null mice.

In summary, an emerging theme of our studies is that SV40 large T antigen has several functional domains that contribute to tumorigenesis, each of which targets different cellular protein(s). Each cell type, in turn, may rely on a different subset of these cellular proteins to maintain the normal balance of differentiated cells. Understanding how viral transforming proteins interfere with these cellular functions will not only provide a better general knowledge of cell growth-regulatory mechanisms, but will also provide a way to probe these mechanisms in normal development and tumorigenesis of distinct cell types *in vivo*. Here we show that in immature thymic T cells, T antigen functionally inactivates p53, leading to resistance to DNA damage-induced cell death and predisposing the cells to tumorigenesis.

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