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Repression of the *NF1* Gene by Tax May Explain the Development of Neurofibromas in Human T-Lymphotropic Virus Type 1 Transgenic Mice

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In familial neurofibromatosis type 1 (NF1), individuals with a germ line-transmitted *NF1* mutation develop multiple neurofibromas. To explain the observation that transgenic mice expressing the human T-lymphotropic virus type 1 (HTLV-1) *tax* gene under the control of the viral regulatory element also develop multiple neurofibromas, we demonstrate that the Tax *trans*-regulator can functionally repress *NF1* gene expression through a *cis*-acting element located immediately upstream of its transcriptional start site, thereby allowing the development of benign neurofibromas without the need for direct mutations in *NF1*. We propose that such a mechanism would suffice to epigenetically alter *NF1* gene expression. The fact that transgenic animals have localized rather than diffuse neurofibroma formation, however, suggests that additional genetic or epigenetic events may be required for neurofibroma formation.

While there is overwhelming evidence for the accumulation of specific genetic mutations in human cancers (45), fundamental questions arising from such findings remain unanswered. First, it is not clear which of these genetic alterations play a role in initiating the disease process. Second, it is uncertain whether they occur entirely at random or are secondary to existing selective pressure. There is now increasing evidence that epigenetic changes, such as imprinting, result in altered gene expression and may contribute significantly to the development and progression of human cancers (18, 40). An interplay between epigenetic and genetic events may be responsible for the multistage progression of disease, a hallmark of most malignancies (36).

Neurofibromatosis type 1 (NF1) is a common hereditary syndrome predisposing to neoplasia (31). It is an autosomal dominant disorder caused by mutations in the *NF1* tumor suppressor gene (7, 42, 44), which encodes a GTPase-activating protein named neurofibromin (2, 25, 46). In familial cases, individuals who inherit a mutated *NF1* allele develop multiple neurofibromas, which are benign tumors of the peripheral nerves. The finding that most individuals predisposed to NF1 develop neurofibromas in childhood or adolescence, but only about 4% develop neurofibrosarcomas later in life (32) (usually arising in deeply placed "plexiform" neurofibromas), suggests that they represent two successive stages of tumor progression. If the malignant tumors were derived from the benign lesions, then there must have been additional genetic or epigenetic changes in the neurofibromas which resulted in progression to neurofibrosarcoma.

Prior to the identification of the *NF1* gene, we reported that insertion of the human T-lymphotropic virus type 1 (HTLV-1) *trans*-regulatory gene (*tax*) under the control of the viral regulatory element (long terminal repeat) into the germline of mice leads to the development of multiple neurofibromas in the resulting transgenic animals (14, 19, 27) (Fig. 1A). This observation has since been confirmed by others (28). The transgenic mouse model has numerous similarities to human NF1. The benign tumors are derived from nerve sheaths and involve both neurofibroblasts and Schwann cells. On occasion, some of the tumors acquire malignant features. Additionally, iris proliferation and adrenal medullary tumors that resemble iris hamartomas and pheochromocytomas in NF1 patients are detected. Since the viral Tax protein is a transcriptional *trans*-regulator which can both activate and repress cellular genes (for review, see reference 48), we speculated that the *tax* transgenic mice might develop peripheral nerve tumors as a result of specific down-regulation of *NF1* gene expression.

For Tax to be capable of regulating *NF1* expression, it must be expressed in the same cell type in which neurofibromin is expressed and from which neurofibromas are derived. To facilitate single-cell analysis, promoter specificity was compared in transgenic mice by using the transcriptional regulatory sequences from the respective genes to drive expression of a reporter gene encoding the bacterial enzyme β-galactosidase (β-Gal). The β-Gal activity could be detected with ease and sensitivity, either in wet whole mounts or in fixed tissue sections.

In transgenic mice carrying both the pLTR-*tax* and pLTR-β*gal* genes, in which the former provided the Tax transactivator to upregulate its own expression as well as that of the latter (3), β-Gal expression was detected in a few neuronal cell bodies within a nerve ganglion (Fig. 1B) and in a majority of cells in a nerve trunk (Fig. 1C). In transgenic mice with the pNF-β*gal* gene, which contains an ~3.8-kb 5′ flanking sequence from the human *NF1* gene, β-Gal activity was detected in a majority of cells within a nerve ganglion (Fig. 1D) and in a few cells in a nerve trunk (Fig. 1E). In adult mice, these are the predominant sites of *NF1* gene expression. The latter finding agrees well with those from immunohistochemical studies which localized neurofibromin accumulation to the same restricted sites (8). Since neurofibromas are derived from peripheral nerve sheaths which are made up of mostly Schwann cells and neurofibroblasts, the colocalization of *tax* and *NF1* expression to cells in peripheral nerves is consistent with the suggestion that the Tax protein may regulate *NF1* transcription.

To address whether Tax may down-regulate *NF1* gene expression, we proceeded to determine by reverse transcriptase

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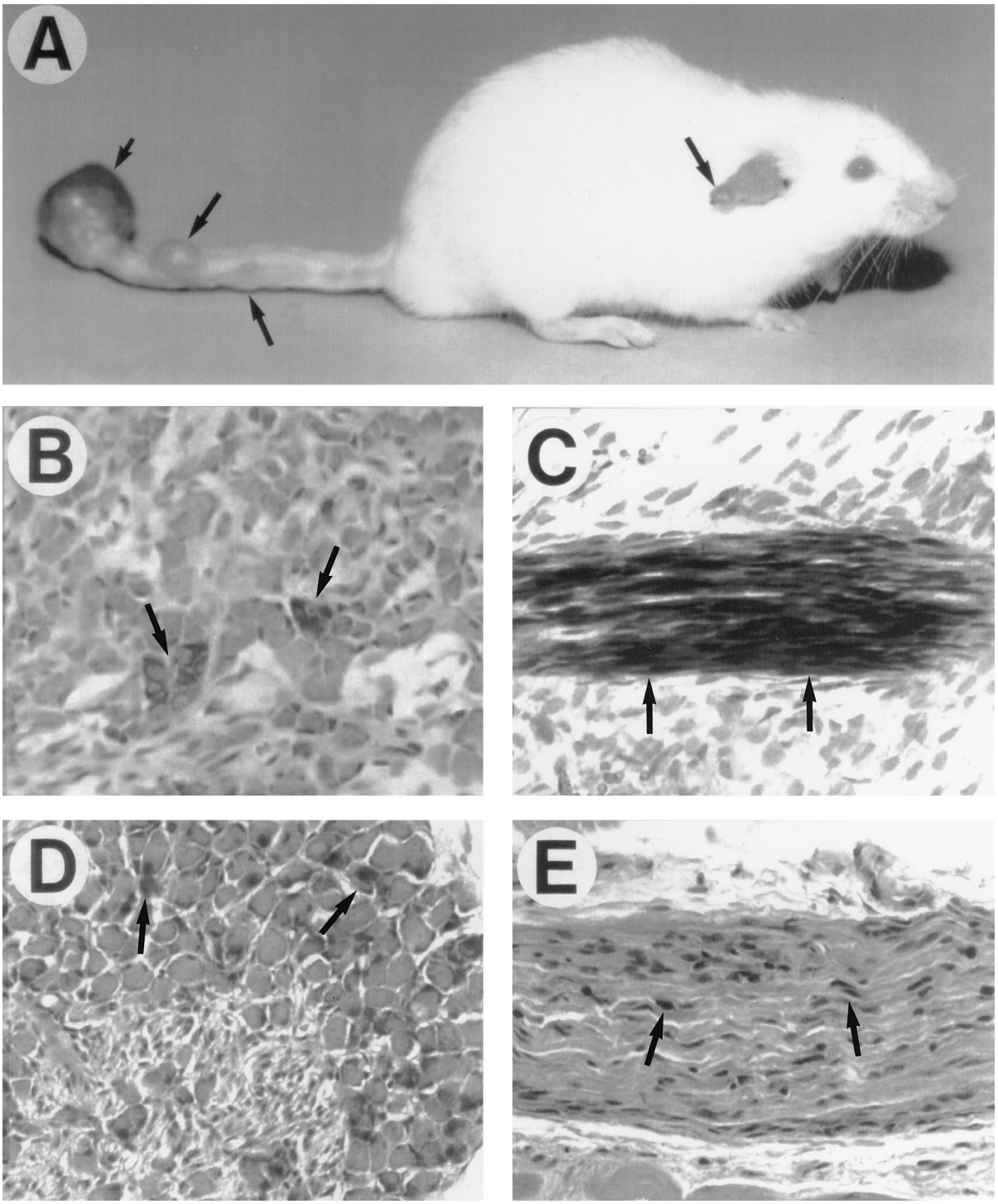

FIG. 1. Gross features of a 3-month-old transgenic mouse carrying the pLTR-*tax* gene and showing multiple neurofibromas (long arrows) and a malignant neurofibrosarcoma (short arrow) in the ear and tail (A). Sites of expression of the *tax* gene in a 2-week-old double transgenic mouse with both the pLTR-*tax* gene and the pLTR-β-*gal* gene, in which basal expression of Tax led to transactivation of both transgenes, were determined. Sections of a nerve ganglion (B) and a nerve trunk (C) are shown. The promoter specificity of the *NF1* gene was similarly determined by analysis of a 2-week-old transgenic mouse carrying the pNF-β*gal* gene. The β-Gal activity in a nerve ganglion (D) and in a nerve trunk (E) is shown. To determine the pattern of β-Gal activity, animals were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline followed by perfusion with a solution containing 5-bromo-4 chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 0.5 mg/ml, 25 mM potassium ferrocyanide, 25 mM potassium ferricyanide, 2 mM $MgCl_2$, 1 mM spermidine, 0.02% Nonidet P-40, and 0.01% sodium deoxycholate (4). Dissected tissues were then incubated at 37°C for 16 h in the X-Gal cocktail, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

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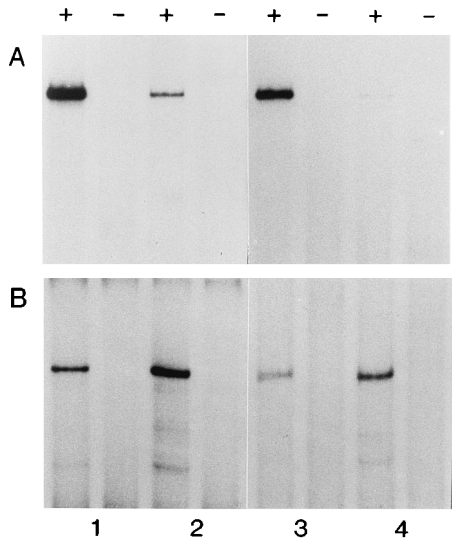

FIG. 2. Analysis of expression of the transgenic *tax* gene and the endogenous *NF1* gene in mice of different ages. Total RNA was extracted from the trigeminal ganglia and the associated nerves of two 3-week-old (lanes 1 and 3) and two 17-week-old (lanes 2 and 4) transgenic mice. The levels of expression of the NF1 mRNA (A) and the Tax mRNA (B) were separately determined by PCR either with (+) or without (−) inclusion of reverse transcriptase (49). Two mice were used for each time point to ensure that the level of gene expression detected was reproducible. The primers specific for NF1 were 5′-GAGTTTGATCAACGAA TTCTTTATG-3′ and 5′TGGCTCTGCAGTGCAGGAGGGTAAG-3′, and those specific for Tax were 5′CTGTCCAGAGCATCAGATCACCTGG-3′ and 5′-GGTTCCATGTATCCATTTCGGAAGG-3′. The 401-bp NF1 PCR product and the 222-bp Tax PCR product were separately detected by hybridization to ^{32}P-end-labelled oligonucleotide probes specific for NF1 (5′-GGCTTGTGGCG GTTTGCAGGACCG-3′) and Tax (5′-CCCAGAGAACCTCTAAGACCCTCA AGGTCCTTAC-3′) followed by electrophoresis in 5% polyacrylamide gels.

PCR analysis whether NF1 mRNA accumulation in nerves is reduced in the Tax transgenic mice. We observed that in the trigeminal nerve, individual control CD1 mice expressed an equivalent level of NF1 transcripts relative to actin mRNA, regardless of whether the mice were young or old (data not shown). In contrast, the relative level of NF1 transcripts in the Tax transgenic mice, while normal at 3 weeks of age became significantly reduced by 17 weeks of age (Fig. 2A) when *tax* expression became elevated (Fig. 2B). We have chosen to analyze the trigeminal nerve because it can be easily dissected and tumors have frequently been found associated with it (19). Given the relatively low frequency of cells in the nerve sheath which normally support significant levels of *NF1* expression, we have purposely chosen to use an averaging assay rather than a single-cell assay in this analysis. Since there were many more Tax-expressing cells than there were NF1-expressing cells in the nerve trunk (Fig. 1), the significant reduction in the accumulation of NF1 transcripts is consistent with the ability of Tax to modulate *NF1* gene expression.

The 5′ flanking region of the *NF1* gene is highly conserved between humans and mice (17). Like typical housekeeping genes, it is highly G+C rich and has no obvious TATA or CCAAT boxes. To determine whether Tax can act, directly or indirectly, on the NF1 promoter region to regulate its expression, we have used the human 3.8-kb 5′ flanking sequence to drive expression of chloramphenicol acetyltransferase (CAT) (pNFS*cat*) in transfection studies, in either the absence or presence of pLTR-*tax*, which encodes the Tax protein. We have chosen to use the NIH 3T3 mouse embryo fibroblast cell line as the recipient in these transfection experiments because we have previously shown that neurofibroblasts in the nerve sheaths of the Tax transgenic mice can support pLTR-*tax* expression (19), and others have observed that NIH 3T3 cells constitutively express the NF1 gene product (9).

Unlike the simian virus 40 promoter, which was nonresponsive to Tax, the 3.8-kb NF1 promoter was markedly inhibited by Tax (Fig. 3). In experiments with various input ratios of pNFS*cat* to pLTR-*tax*, we observed that inhibition of NF1 transcriptional activity was dependent on the amount of Tax protein made (data not shown). Under optimal conditions, >92% inhibition could be achieved. This suggests that there must be a *cis*-acting element(s) located somewhere within the 3.8-kb fragment used which can be regulated by Tax to control *NF1* gene expression.

In order to map the putative *cis*-acting element within the NF1 promoter region, we have performed deletional analysis by scoring for the loss of Tax responsiveness in transfection experiments. Results from selected mutations are shown (Fig. 3). It is notable that certain deletions resulted in either an increase or a decrease in basal promoter activity in the absence of Tax, suggesting that there are both negative and positive control elements at different regions within this promoter fragment which contribute to normal *NF1* expression. For example, there appear to be an inhibitory control element(s) located between positions −1663 and −995 and multiple stimulatory control elements lying between −995 and −3. Despite the presence of these regulatory elements, we were able to demonstrate that overlapping deletions from −2901 to −236 did not interfere with Tax repression. However, deletion downstream of −236 and up to −3 could suffice to completely ablate the ability of Tax to down-regulate this promoter.

The ability of Tax to down-regulate the human NF1 promoter in NIH 3T3 cells and the high level of homology between the human and mouse promoter sequences prompted us to determine whether stable transfection of the *tax* gene into NIH 3T3 cells would lead to down-regulation of the endogenous mouse *NF1* gene as shown by a decrease in steady-state accumulation of its gene product. Neurofibromin has been shown to be constitutively expressed in NIH 3T3 cells (9). Selective inhibition of its synthesis upon introduction of the *tax* gene would constitute proof of transregulation of expression and confirm our observation with transgenic mice that increased expression of Tax correlated with a substantial decrease in NF1 transcripts in nerves.

The pLTR-*tax* gene was cotransfected with the pRSV*neo* gene into NIH 3T3 cells, and G418-resistant clones were isolated. The presence of the *tax* gene was confirmed by radioimmunoprecipitation analysis of metabolically labelled cell extracts with an anti-Tax serum (19, 27). When the parental NIH 3T3 cells and a G418-resistant *neo*-only-transfected clone were compared with two independent G418-resistant *neo*- and *tax*-transfected clones, the 40-kDa Tax protein was detected only in the latter (Fig. 4A), which were selected because of their difference in the level of expression of the transfected gene.

When the same set of radiolabelled cell extracts were subjected to immunoprecipitation with an anti-NF1 serum raised against the catalytic region of neurofibromin (9), a substantial reduction (~90%) in the amount of the ~220-kDa protein was detected in the two Tax-expressing clones compared with that in the two control cell lines (Fig. 4B). The clone that expressed a high level of the Tax protein has a somewhat greater reduction of neurofibromin. All four radiolabelled cell extracts had virtually identical specific activities and, when equivalent amounts of protein from each were analyzed without immunoprecipitation, showed overall protein patterns that were practically indistinguishable from one another (Fig. 4C).

Our findings with tissue culture cells that (i) a stably trans-

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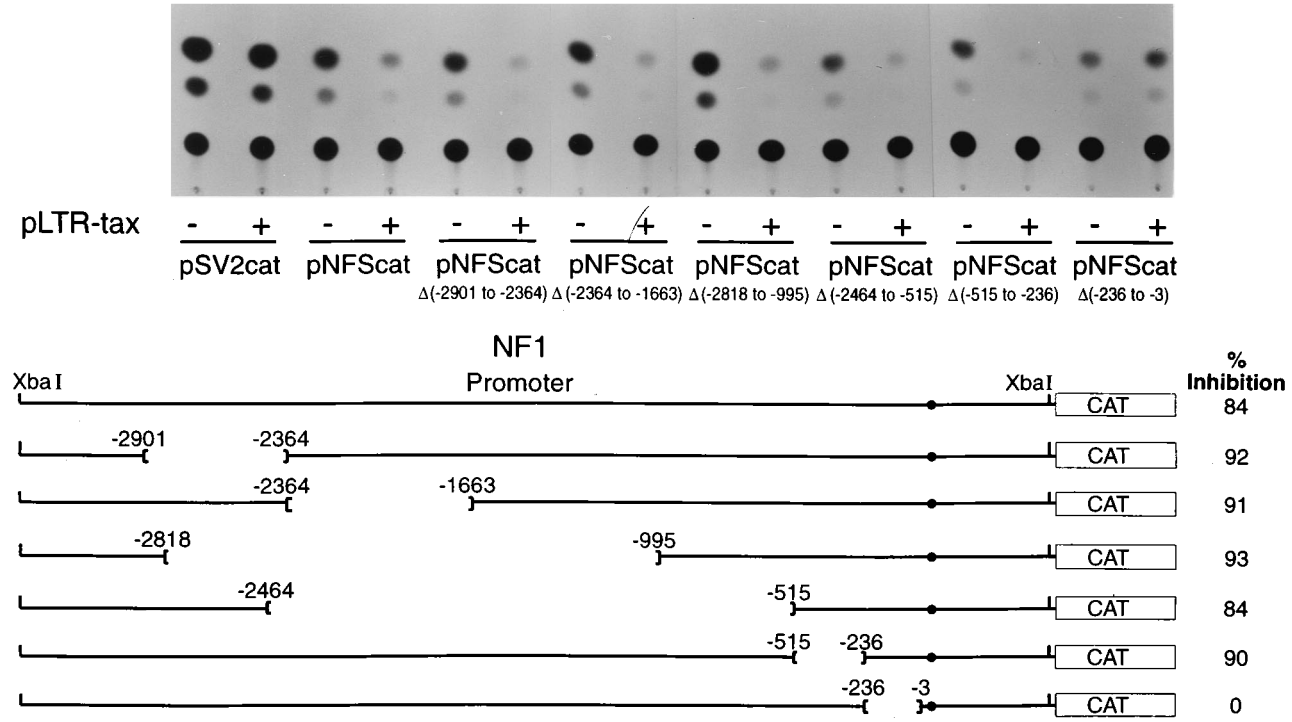

FIG. 3. Dissection of the NF1 promoter region for Tax responsiveness. The 3.8-kb 5′ flanking sequence of the *NF1* gene was excised from the plasmid 17L1B[20] as an *Xho*I-*Sst*II fragment and inserted into the promotorless CAT reporter construct pCAT3M at the *Xba*I site. The resulting plasmid, pNFS*cat*, was subjected to deletions at the restriction sites *Nde*I to *Sap*I (−2901 to −2364), *Sap*I to *Xcm*I (−2364 to −1663), *Tth*III1 to *Tth*III1 (−2818 to −995), *Stu*I to *Stu*I (−2464 to −515), *Stu*I to *Bss*HI (−515 to −236), and *Bss*HI to *Eco*NI (−236 to −3). The number coordinates correspond to the locations of the restriction sites with respect to the NF1 transcriptional start site (●). A simian virus 40 promoter-bearing *cat* construct (pSV2*cat*) was used as a control. The transcriptional activities of these constructs were determined by CAT assays (13). Briefly, 10 μg of wild-type or deletion mutant NF1 DNA construct was transfected with either 20 μg of filler plasmid DNA (−) or 20 μg of pLTR-*tax* DNA (+). After 48 h, cell extracts were prepared and incubated with ^{14}C-chloramphenicol, and the extent of acetylation was determined by thin-layer chromatography. The results for each of the constructs, in either the absence or presence of Tax, are indicated as percentages of inhibition of CAT activity.

fected *tax* gene could significantly reduce the level of the *NF1* gene product and (ii) the *tax* gene in transient transfection experiments could down-regulate transcription of the NF1 promoter through a specific target site, explain our observations with transgenic mice that (i) both the *tax* gene and the *NF1* gene were transcriptionally active in cells in the peripheral nerve sheath where neurofibromas were derived and that (ii) an increase in *tax* expression with age correlated with a decrease in *NF1* gene expression and the development of numerous neurofibromas. Together, our observations suggest that Tax can repress the *NF1* promoter, leading to the development of benign tumors in peripheral nerves.

Since its isolation 15 years ago, HTLV-1 has been associated with an increasing number of disorders, including adult T-cell leukemia (20, 29), tropical spastic paraparesis (12, 34), infective dermatitis (24), hypercaliuria and end-stage hypercalcemia (6, 11), Sjögren's syndrome (37, 41), and polymyositis (22, 26, 38). In each of these disorders, the role of HTLV-1 in the disease process has not been defined. Indeed, while HTLV-1 can be transmitted by cell fusion to a variety of cell types in culture, viral gene expression in infected patients has been difficult to demonstrate. Even leukemic cells isolated from individuals with ATL do not usually support viral gene expression (21). The transgenic approach appears uniquely appropriate for both identifying the target cells and delineating the disease mechanisms (15, 16, 35).

The present study suggests that the *tax* gene of HTLV-1 may act as a cofactor for the development of neurofibromas and prompts an epidemiological study to determine whether there is such an association in areas in which HTLV-1 is endemic. It has to be pointed out that most of the disorders associated with HTLV-1 are far more widespread than originally suspected and that clinical symptoms frequently develop only after a long latency and with a low penetrance. This may reflect a low frequency of infection of the respective target cells and the indirect nature of the viral function in inducing the pathological changes, as shown here for the development of neurofibromas.

Interestingly, our finding that physical trauma can function as a cofactor for Tax transactivation of the HTLV-1 long terminal repeat in epidermal keratinocytes in transgenic mice led to our demonstration that Tax is expressed in the hyperplastic epidermis of infective dermatitis in HTLV-1-infected patients (our unpublished data). We are hopeful that we will similarly be able to show *tax* gene expression in HTLV-1-positive NF lesions. However, we anticipate that it may be difficult to demonstrate down-regulation of *NF1* gene expression in neurofibroma cells from patients because their origin and differentiation state are still being debated, which makes it difficult to compare the levels of neurofibromin expression in uninvolved and involved cells of the same genealogy. An alternative approach would be to try culturing these benign NF cells and determine whether blockage of *tax* gene expression, possibly by using antisense oligonucleotides, would lead to derepression of *NF1* and suppression of cell growth.

Mapping of the Tax-responsive element of the NF1 promoter to a region of 233 bp is preliminary but informative. While this region does not have a TATA box, it has both a

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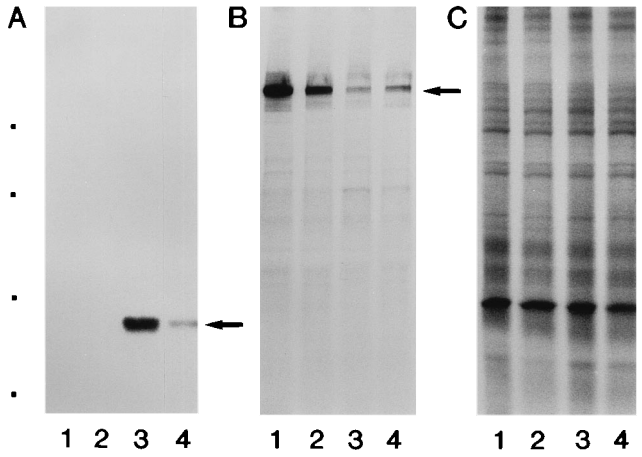

FIG. 4. Analysis of neurofibromin expression in cells stably transfected with the *tax* gene. Cell extracts were immunoprecipitated with either a rabbit anti-Tax antibody (A) or a rabbit anti-NF1 antibody (B). As a control, an equivalent amount of each extract was also analyzed without immunoprecipitation (C). The cells used were nontransfected NIH 3T3 cells (lanes 1); a *neo*-transfected clone, B1 (lanes 2); and two *neo*- and *tax*-transfected clones, A9 and C3 (lanes 3 and 4, respectively). Transfections were performed with 20 μg of pLTR-*tax* and 2 μg of pRSV*neo*. G418-resistant colonies were picked and expanded. Subconfluent cultures of appropriate cell clones were metabolically labelled with [^{35}S]methionine and [^{35}S]cysteine for 5 h. Cells were rinsed with phosphate-buffered saline and lysed with a buffer containing 50 mM Tris HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate, and 1.0% Triton X-100. Equivalent amounts of protein from each cell line were immunoprecipitated with the appropriate antibody. The immunoprecipitate was electrophoresed on a 10% polyacrylamide–sodium dodecyl sulfate gel. The arrows indicate the positions of the 40-kDa Tax protein and the 220-kDa neurofibromin. The molecular mass markers used have apparent molecular masses of 200,000, 97,000, 45,000, and 30,000 Da.

cyclic AMP (cAMP) response element and a serum response element that are perfectly conserved between the mouse and human genes (17). Whether either of these elements is involved in the repression is unclear, but the cAMP response element is of particular interest because Tax has been shown to directly increase the DNA binding activities of multiple cAMP response element binding transcription factors, including CREB, ATF-1, and ATF-2, by promoting their dimerization to form either homo- or heterodimers (1, 10, 39, 43, 47, 50). The suggestion that a particular transcription regulator can both activate and repress gene expression is intriguing (30, 33). Depending on the regulatory subunit, a specific DNA binding protein can have diametric consequences. Alternatively, the molar ratio between the two may suffice to dictate which opposing function will predominate. Tax may be such a regulator.

Since the stably transfected *tax* gene could repress the endogenous *NF1* gene, we are currently pursuing a more precise mapping of the Tax-responsive element within the chromosomal NF1 promoter region rather than that in a cotransfected NF1 promoter construct. We are hopeful that this approach will lead to the identification of not only the specific *cis*-acting element but also, eventually, the relevant *trans*-acting proteins.

The apparent excess of Tax-expressing cells over NF1-positive cells in the Tax transgenic mice may predict the development of diffuse neurofibromas at a very early age. Interestingly, we observed predominantly nodular proliferation of cells simultaneously at numerous body sites, eventually killing the mouse at a premature age. This could imply the need for an additional genetic event. Alternatively, *tax* gene expression in the rare NF1-positive cells may be subthreshold in most cells and hence may not suffice to completely shut off the NF1 tumor suppressor activity. Indeed, the transgenic line with the highest level of *tax* gene expression has the shortest latency for tumor development and is the earliest to succumb to tumors.

Analysis of mice with a targeted mutation in the *NF1* gene has revealed that *NF1*$^{+/-}$ heterozygotes, while predisposed to the formation of various tumor types, do not develop neurofibromas (5, 23). Furthermore, *NF1*$^{-/-}$ homozygote chimeras generated by injection of normal blastocysts with ES cells harboring a double knockout of the *NF1* gene only occasionally develop neurofibromas, which are invariably focal in nature. These findings suggest that the development of neurofibromas in mice requires not only the loss of both *NF1* alleles but also additional events, either genetic or epigenetic. This is consistent with our observation that repression of *NF1* gene expression by Tax, a process which is expected to affect both *NF1* alleles, while sufficient to induce neurofibromas gave rise to multifocal rather than diffuse tumors. These transgenic and knockout studies with mice further our understanding of the mechanism which underlies neurofibroma formation.

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REFERENCES

1. **Adya, N., L.-J. Zhao, W. Huang, I. Boros, and C.-Z. Giam.** 1994. Expansion of CREB's DNA recognition specificity by Tax results from interaction with Ala-Ala-Arg at positions 282–284 near the conserved DNA-binding domain of CREB. Proc. Natl. Acad. Sci. USA **91:**5642–5646.
2. **Ballester, R., D. Marchuk, M. Boguski, A. Saulino, R. Letcher, M. Wigler, and F. Collins.** 1990. The *NF1* locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell **63:**851–859.
3. **Bieberich, C. J., C. M. King, B. T. Tinkle, and G. Jay.** 1993. A transgenic model of transactivation by the Tax protein of HTLV-1. Virology **196:**309–318.
4. **Bieberich, C. J., M. F. Utset, A. Awgulewitsch, and F. H. Ruddle.** 1990. Evidence for positive and negative regulation of the *Hox-3.1* gene. Proc. Natl. Acad. Sci. USA **87:**8462–8466.
5. **Brannan, C. I., A. S. Perkins, K. S. Vogel, N. Ratner, M. L. Nordlund, S. W. Reid, A. M. Buchberg, N. A. Jenkins, L. F. Parada, and N. G. Copeland.** 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev. **8:**1019–10291.
6. **Catovsky, D., M. F. Greaves, M. Rose, D. A. Galton, A. W. G. Goolden, D. R. McCluskey, J. M. White, I. Lampert, G. Bourikas, R. Ireland, A. I. Brownell, J. M. Bridges, W. A. Blattner, and R. C. Gallo.** 1982. Adult T-cell lymphoma-leukemia in blacks from the West Indies. Lancet **i:**639–643.
7. **Cawthon, R. M., R. Weiss, G. Xu, D. Viskochil, M. Culver, J. Stevens, M. Robertson, D. Dunn, R. Gesteland, P. O'Connell, and R. White.** 1990. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell **62:**193–201.
8. **Daston, M. M., H. Scrable, M. Nordlund, A. K. Sturbaum, L. M. Nissen, and N. Ratner.** 1992. The protein product of the neurofibromatosis type 1 gene is expressed at highest abundance in neurons, Schwann cells, and oligodendrocytes. Neuron **8:**415–428.
9. **DeClue, J. E., B. D. Cohen, and D. R. Lowy.** 1991. Identification and characterization of the neurofibromatosis type 1 protein product. Proc. Natl. Acad. Sci. USA **88:**9914–9918.
10. **Fujii, M., H. Tsuchiya, T. Chuhjo, T. Akizawa, and M. Seiki.** 1992. Interaction of HTLV-1 Tax1 with p67SRF causes the aberrant induction of cellular immediate early genes through CArG boxes. Genes Dev. **6:**2066–2076.
11. **Fukumoto, S., T. Matsumoto, K. Ikeda, T. Yamashita, et al.** 1988. Clinical evaluation of calcium metabolism in adult T-cell leukemia/lymphoma. Arch. Intern. Med. **148:**921–925.
12. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. De Thé.** 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet **ii:**407–410.
13. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
14. **Green, J. E., A. M. Baird, S. H. Hinrichs, G. K. Klintworth, and G. Jay.** 1992. Adrenal medullary tumors and iris proliferation in a transgenic mouse model of neurofibromatosis. Am. J. Pathol. **140:**1401–1410.
15. **Green, J. E., C. G. Begley, D. K. Wagner, T. A. Waldmann, and G. Jay.** 1989. Transactivation of granulocyte-macrophage colony-stimulating factor and the interleukin-2 receptor in transgenic mice carrying the human T-lymphotropic virus type 1 *tax* gene. Mol. Cell. Biol. **9:**4731–4737.

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16. **Green, J. E., S. H. Hinrichs, J. Vogel, and G. Jay.** 1989. Exocrinopathy resembling Sjögren's syndrome in HTLV-1 *tax* transgenic mice. Nature (London) **341:**72–74.
17. **Hajra, A., A. Martin-Gallardo, S. A. Tarlé, M. Freedman, S. Wilson-Gunn, A. Bernards, and F. S. Collins.** 1994. DNA sequences in the promoter region of the *NF1* gene are highly conserved between human and mouse. Genomics **21:**649–652.
18. **Herman, J. G., F. Latif, Y. Weng, M. I. Lerman, B. Zbar, S. Liu, D. Samid, D.-S. R. Duan, J. R. Gnarra, W. M. Linehan, and S. B. Baylin.** 1994. Silencing of the *VHL* tumor-suppressor gene by DNA methylation in renal carcinoma. Proc. Natl. Acad. Sci. USA **91:**9700–9704.
19. **Hinrichs, S. H., M. Nerenberg, R. K. Reynolds, G. Khoury, and G. Jay.** 1987. A transgenic mouse model for human neurofibromatosis. Science **237:**1340–1343.
20. **Hinuma, Y., K. Nagata, M. Hanaoka, M. Nakai, T. Matsumoto, K.-I. Kinoshita, S. Shirakawa, and I. Miyoshi.** 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc. Natl. Acad. Sci. USA **78:**6476–6480.
21. **Imada, K., A. Takaori-Kondo, T. Akaji, K. Shimotohono, K. Sugamura, T. Hattori, H. Yamabe, M. Okuma, and T. Uchiyama.** 1995. Tumorigenicity of human T-cell leukemia virus type I-infected cell lines in severe combined immunodeficient mice and characterization of the cells proliferating in vivo. Blood **86:**2350–2357.
22. **Ishii, K., K. Yamato, Y. Iwahara, T. Eguchi, et al.** 1991. Isolation of HTLV-I from muscle of a patient with polymyositis. Am. J. Med. **90:**267–269.
23. **Jacks, T., T. S. Shih, E. M. Schmitt, R. T. Bronson, A. Bernards, and R. A. Weinberg.** 1994. Tumour predisposition in mice heterozygous for a targeted mutation in *NF1*. Nat. Genet. **7:**353–361.
24. **LaGrenade, L., B. Hanchard, V. Fletcher, B. Cranston, and W. Blattner.** 1990. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. Lancet **336:**1345–1347.
25. **Martin, G. A., D. Viskochil, G. Bollag, P. C. McCabe, W. J. Crosier, H. Haubruck, L. Conroy, R. Clark, P. O'Connell, R. M. Cawthon, M. A. Innis, and F. McCormick.** 1990. The GAP-related domain of the neurofibromatosis type 1 gene product interacts with *ras* p21. Cell **63:**843–849.
26. **Mora, C. A., R. M. Garruto, P. Brown, D. Guiroy, et al.** 1988. Seroprevalence of antibodies to HTLV-I in patients with chronic neurological disorders other than tropical spastic paraparesis. Ann. Neurol. **23:**S192–S195.
27. **Nerenberg, M., S. H. Hinrichs, R. K. Reynolds, G. Khoury, and G. Jay.** 1987. The *tat* gene of human T-lymphotropic virus type I induces mesenchymal tumors in transgenic mice. Science **237:**1324–1329.
28. **Pavlakis, G. N., B. K. Felber, V. Ciminale, T. Unge, L. Solomin, and J. E. Harrison.** 1992. Structure, regulation and oncogenic mechanisms of HTLV-I and HTLV-II. Leukemia **3:**176S–180S.
29. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77:**7415–7419.
30. **Pollock, R., G. Jay, and C. J. Bieberich.** 1992. Altering the pattern of *Hox 3.1* expression: evidence for antipodal gene regulation. Cell **71:**911–924.
31. **Riccardi, V. M.** 1981. Von Recklinghausen neurofibromatosis. N. Engl. J. Med. **305:**1617–1627.
32. **Riccardi, V. M.** 1991. Neurofibromatosis: phenotype, natural history, and pathogenesis, 2nd ed. Johns Hopkins University Press, Baltimore.
33. **Roberts, S. G. E., and M. R. Green.** 1995. Dichotomous regulators. Nature (London) **375:**105–106.
34. **Rodgers-Johnson, P., D. C. Gajdusek, O. St. C. Morgan, V. Zaninovic, P. S. Sarin, and O. S. Graham.** 1985. HTLV-I and HTLV-III antibodies and tropical spastic paraparesis. Lancet **ii:**1247–1248.
35. **Ruddle, N. H., C.-B. Li, C. H. William, P. Santiago, N. Troiano, G. Jay, M. Horowitz, and R. Baron.** 1993. Mice transgenic for HTLV-1 LTR-*tax* exhibit Tax expression in bone, skeletal alterations, and high bone turnover. Virology **197:**196–204.
36. **Scrable, H. J., C. Sapienza, and W. K. Cavenee.** 1990. Genetic and epigenetic losses of heterozygosity in cancer predisposition and progression. Adv. Cancer Res. **54:**25–62.
37. **Smith, F. B., H. Rajdeo, N. Panesar, K. Bhuta, and R. Stahl.** 1988. Benign lymphoepithelial lesion of the parotid gland in intravenous drug users. Arch. Pathol. Lab. Med. **112:**742–745.
38. **Sowa, J. M.** 1992. Human T lymphotropic virus I, myelopathy, polymyositis and synovitis: an expanding rheumatic spectrum. J. Rheumatol. **19:**316–318.
39. **Suzuki, T., J.-I. Fujisawa, M. Toita, and M. Yoshida.** The trans-activator Tax of human T-cell leukemia virus type 1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. Proc. Natl. Acad. Sci. USA **90:**610–614.
40. **Ueda, H., S. J. Ullrich, J. D. Gangemi, C. A. Kappel, L. Ngo, M. A. Feitelson, and G. Jay.** 1995. Functional inactivation but not structural mutation of p53 causes liver cancer. Nat. Genet. **9:**41–47.
41. **Vernant, J.-C., G. Buisson, J. Magdeleine, J. De Thore, A. Jouannelle, C. Neisson-Vernant, and N. Monplaisir.** 1988. T-lymphocyte alveolitis, tropical spastic paresis, and Sjögren syndrome. Lancet **i:**177.
42. **Viskochil, D., A. M. Buchberg, G. Xu, R. M. Cawthon, J. Stevens, R. K. Wolff, M. Culver, J. C. Carey, N. G. Copeland, N. A. Jenkins, R. White, and P. O'Connell.** 1990. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell **62:**187–192.
43. **Wagner, S., and M. R. Green.** 1993. HTLV-I Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization. Science **262:**395–399.
44. **Wallace, M. R., D. A. Marchuk, L. B. Andersen, R. Letcher, H. M. Odeh, A. M. Saulino, J. W. Fountain, A. Brereton, J. Nicholson, A. L. Mitchell, B. H. Brownstein, and F. S. Collins.** 1990. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science **249:**181–186.
45. **Weinberg, R. A.** 1991. Tumor suppressor genes. Science **254:**1138–1146.
46. **Xu, G., B. Lin, K. Tanaka, D. Dunn, D. Wood, R. Gesteland, R. White, R. Weiss, and F. Tamanoi.** 1990. The catalytic domain of the neurofibromatosis type 1 gene product stimulates *ras* GTPase and complements *ira* mutants of S. cerevisiae. Cell **63:**835–841.
47. **Yin, M.-J., E. J. Paulssen, J.-S. Seeler, and R. B. Gaynor.** 1995. Protein domains involved in both in vivo and in vitro interactions between human T-cell leukemia virus type I Tax and CREB. J. Virol. **69:**3420–3432.
48. **Yoshida, M.** 1993. HTLV-1 Tax: regulation of gene expression and disease. Trends Microbiol. **1:**131–135.
49. **Yoshioka, T., L. Feigenbaum, and G. Jay.** 1991. Transgenic mouse model for central nervous system demyelination. Mol. Cell. Biol. **11:**5479–5486.
50. **Zhao, L., and C. Giam.** 1992. Human T-cell lymphotropic virus type 1 (HTLV-1) transcriptional activator, Tax, enhances CREB binding to HTLV-1 21-base-pair repeats by protein-protein interaction. Proc. Natl. Acad. Sci. USA **89:**7070–7074.

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