Brief Definitive Report

Autonomous Growth and Tumorigenicity Induced by P40/Interleukin 9 cDNA Transfection of a Mouse P40-dependent T Cell Line

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Summary

To test the transforming potential of deregulated P40/Interleukin 9 expression, we transfected a mouse P40-dependent T cell line with P40 cDNA, and examined the tumorigenicity of the resulting transfectants. When the cells, which grew autonomously in vitro, were injected intraperitoneally or subcutaneously into syngeneic mice, a very high tumor incidence was observed with as few as 10^4 cells per inoculum. Animals died as a result of widespread dissemination of lymphomatous tissue to abdominal and thoracic organs. The same P40-dependent cell line transfected with a control construct did not form tumors even after injection of 10^7 cells. These results indicate that uncontrolled expression of P40 can support T cell proliferation in vivo, and may be a transforming event involved in the development of certain T cell tumors.

P40 is a lymphokine produced by activated $CD4^+$ T cells that was originally identified in the mouse as a 30–40 kDa factor capable of supporting the growth of certain T helper clones (1), but not of cytolytic T cell clones or of fresh T cells (2). Subsequently, mouse P40 was found to enhance the growth of bone-marrow-derived mast cell lines in response to IL-3 (3) and that of fetal thymocytes in response to IL-2 (4). Human P40 was shown to stimulate the proliferation of a megakaryoblastic leukemia (5), to cause increased survival of certain T cell lines (6), and to enhance erythroid burst forming activity (7, 8). Considering these multiple activities, it has been proposed that P40 be renamed IL-9 (5).

Recently, it has been shown that IL-2–dependent T cell lines become tumorigenic after transfection with IL-2 cDNA (9, 10), a clear demonstration that aberrant expression of IL-2 can lead to T cell transformation. Here we have followed the same approach to examine the transforming potential of P40.

Materials and Methods

Transfections. Mouse P40 cDNA P40.2B4 (11) was subcloned into the Sal I site of expression vector pBMGneo (12). P40-dependent T cell line TS1 (1) was transfected by electroporation with the resulting sense or antisense constructs. Transfectants were selected in G418 and cloned by limiting dilution.

P40 Assay. The concentration of P40 secreted by transfectants was determined by measuring the growth factor activity of conditioned media for TS1 cells as described (1). 1 U/ml is defined as the concentration required for half-maximal growth. The specificity of the TS1 assay, which registers both P40 and IL-4, was ensured by inhibition with the IgG fraction of a rabbit antiserum raised against purified P40 or with anti-IL-4 mAb 11B11 (13).

Histology. Tissues were fixed in formalin and stained with hematoxylin-eosin.

Results and Discussion

Expression of P40 cDNA in P40-dependent T Cells. The P40-dependent T cell line used in the present work was originally from a IA^b-restricted KLH-specific Th clone by culture in medium conditioned by PMA-stimulated Th cells (1). This cell line, TS1, is entirely dependent on an exogenous source of either P40 or IL-4 for its growth and survival in vitro. TS1 was transfected with a P40 cDNA cloned in expression vector pBMGneo in sense or missense orientation. The cells transfected with the P40-expressing construct (TS1.G6) no longer required exogenous P40 for growth (Fig. 1), whereas the cells transfected with the missense construct (TS1.E10) remained factor-dependent. In the presence of P40, the two cell lines had essentially identical doubling times (11.6 h and 12.3 h for TS1.E10 and TS1.G6, respectively). TS1.G6 cells could be cloned in the absence of exogenous P40. However, addition of P40 increased the cloning efficiency about two-fold, indicating that the cells were still responsive to the factor.

TS1.G6-conditioned medium supported the growth of normal TS1 cells, and this activity was neutralized by the

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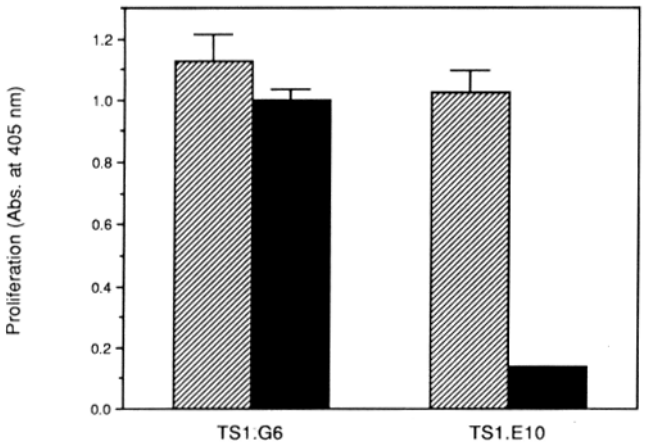

Figure 1. Factor-independent growth of TS1 cells transfected with P40 cDNA. TS1 cells transfected with P40 cDNA cloned in sense (TS1.G6 cells) or missense (TS1.E10) orientation in expression vector pBMGneo were seeded in microwells at a density of 3000 cells/well, and grown in the presence (▨) or absence (■) of P40 (5 U/ml). Cell numbers were evaluated after 3 d by measuring the hexosaminidase content of the wells.

Table 1. *Tumorigenicity of TS1 Cells Transfected with P40 cDNA*

Cells	Size of inoculum	Mice with tumors/mice injected: Normal	Irradiated
TS1.E10	10^7	0/5	0/5
TS1.G6	10^7	5/5 (81)	2/2 (46)
	10^6	6/6 (93)	2/2 (70)
	10^5	5/5 (102)	2/2 (78)
	10^4	5/5 (116)	2/2 (101)
TS1.G6/2	10^6	5/5 (124)	2/2 (85)
TS1.G6/3	10^6	5/5 (102)	2/2 (88)
TS1.G6/5	10^6	4/5 (89)	2/2 (49)
TS1.G6/6	10^6	5/5 (103)	2/2 (46)
TS1.G6/102	10^6	5/5 (124)	2/2 (91)

TS1 transfectants were injected s.c. at the indicated doses into normal or irradiated (600 rads) syngeneic C57BL/6 mice. TS1.E10 and TS1.G6 are the uncloned G418-resistant populations obtained after transfection of TS1 with P40 cDNA cloned in antisense and sense orientation, respectively. TS1.G6/n are individual clones isolated from the TS1.G6 population. Figures in parentheses correspond to mean survival times in days.

IgG fraction of a rabbit antiserum raised against purified P40 (Fig. 2). The specificity of this inhibition was demonstrated by the failure of the antiserum to block the proliferation of TS1 cells induced by IL-4. By reference to a dose-response curve constructed with the recombinant factor, the amount of P40 secreted by TS1.G6 cells was evaluated at ~1 ng/10^6 cells/48 h. That this P40 originated from the transfected cDNA and not from the endogenous gene was demonstrated by the finding that the P40 message in TS1.G6 was larger than normal P40 mRNA. This difference probably results from the formation of a large transcript including the rabbit β globin gene of the vector, as described for IL-2 cDNA expressed from a similar construct (10).

Taken collectively, these observations suggested that TS1.G6 cells functioned via an autocrine P40 loop. However, we failed to block the proliferation of TS1.G6 cells themselves with our rabbit anti-P40 IgG fraction. This result is reminiscent of the observations reported previously for factor-dependent cell lines transfected with cDNA encoding GM-CSF (14), IL-4 (15), or a nonsecreted form of IL-3 targeted to the endoplasmic reticulum by addition of a KDEL motif (16). It suggests that endogenous P40 may bind to P40 receptors intracellularly or reach local concentrations beyond the neutralizing capacity of the antiserum.

Tumorigenicity of P40-transfected T Cell Lines. Injection of

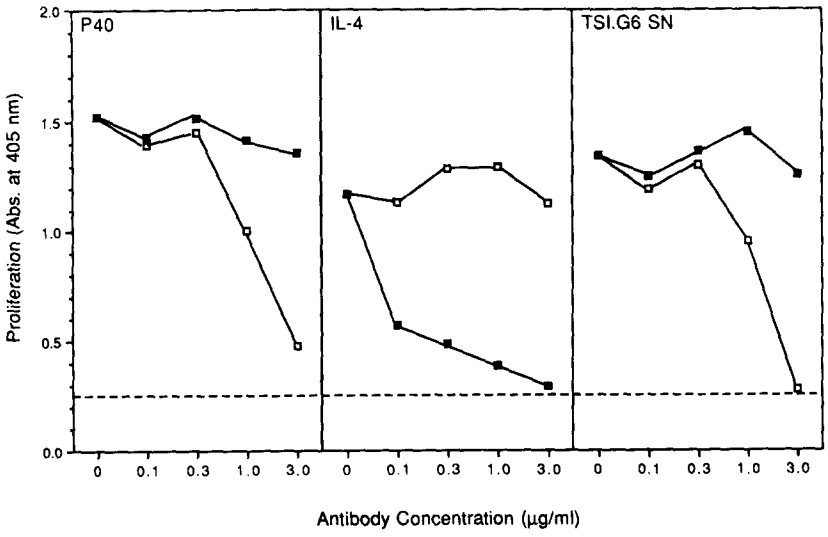

Figure 2. Identification of P40 in medium conditioned by TS1.G6 cells. Factor-dependent TS1 cells were grown with P40 (5U/ml), IL-4 (50 U/ml), or TS1.G6-conditioned medium (10%) in the presence of increasing doses of rabbit anti-P40 IgG (□) or of anti-IL-4 mAb 11B11 (■). The dotted line indicates the background level observed in the absence of growth factor.

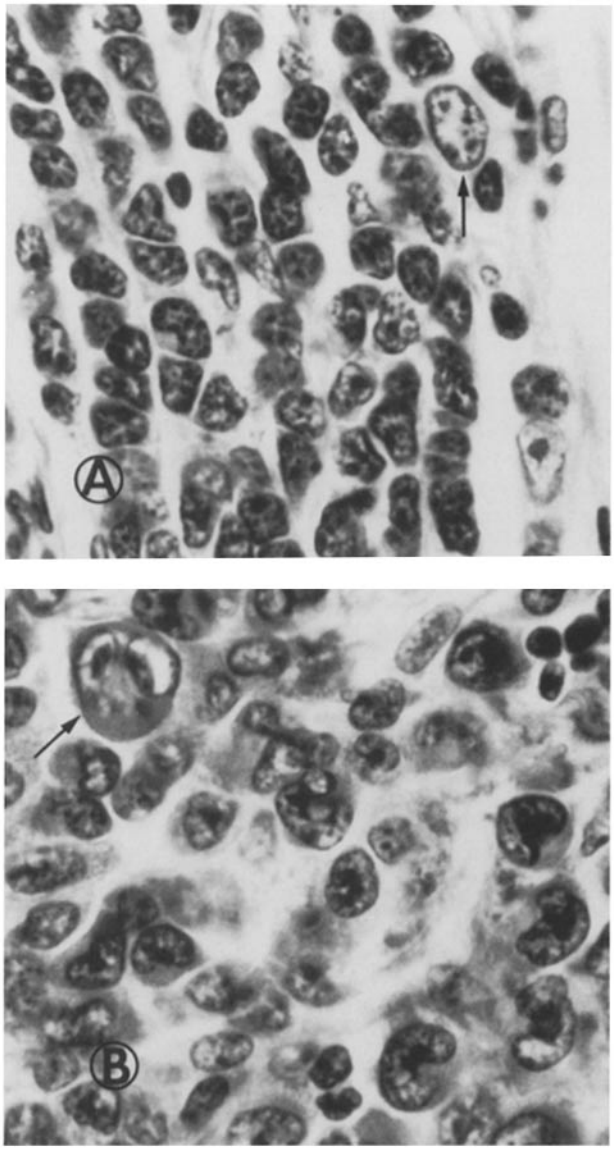

Figure 3. Histology of lymph node infiltrated with TS1.G6 cells. (*A*) Area invaded by predominantly medium-sized neoplastic cells with some large blasts (*arrow*). (*B*) Section showing a majority of large blastic cells with deeply indented nuclei and occasional prominent Golgi apparatus (*arrow*). ×980.

syngeneic C57BL/6 mice with TS1.G6 cells resulted in the development of progressive tumors that killed the animals in 3–4 mo. A 100% tumor incidence was observed with inocula ranging from 10^4 to 10^7 cells (Table 1). In irradiated animals, tumors appeared somewhat earlier than in normal mice, suggesting that TS1.G6 cells elicited a significant immune response in the syngeneic host. In sharp contrast with these results, not a single tumor developed in normal or irradiated mice injected with up to 10^7 cells of the control TS1.E10 line.

The tumorigenicity of TS1.G6 cells was not due to a minor subset of the transfected population because five out of five transfected clones were tumorigenic. Moreover, the possibility that tumors arising in mice injected with TS1.G6 cells could be formed by host cells in response to P40 was ruled out by analysis of T cell receptor gene rearrangements in the tumoral tissue and by the finding that cells recovered from a TS1.G6 tumor were G418-resistant (data not shown).

Histological examination of the mice injected with TS1.G6 cells showed the presence of lymphomatous tissue infiltrating not only lymph nodes but also abdominal and thoracic organs, including lungs and thymus. In the lymph nodes, the neoplastic cells invade the pulp around the sinuses leaving only a few residual islands of small lymphocytes. In some areas, the tumor cells are predominantly of medium size with occasional large blasts (Fig. 3 *A*), whereas, in other areas, all cells are large and blastic with indented or lobulated nuclei (Fig. 3 *B*). The same differences are observed within clonal TS1.G6 cultures and are characteristic of the TS1 cell line itself.

Conclusion. Our results show that P40 can act as a T cell growth factor in vivo. Similar results have been obtained before with IL-2 (9, 10) but not so far with IL-4 (15), possibly because of the potent anti-tumor reaction elicited by IL-4 transfectants in vivo (17). The observation that a P40-dependent T cell line becomes tumorigenic after transfection with P40 cDNA suggests that inappropriate expression of the P40 gene could be involved in the development of T cell tumors. Analysis of P40 expression in T cell neoplasia or in other hematopoietic tumors will now be required to evaluate the importance of this phenomenon.

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