AUTOCRINE GROWTH AND TUMORIGENICITY OF INTERLEUKIN 2-DEPENDENT HELPER T CELLS TRANSFECTED WITH IL-2 GENE

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An aberrant operation of the autocrine self-stimulation has been postulated to be one of the mechanisms leading cells to malignant transformation (1–3). Cells could escape from normal growth control and become malignant by endogenous production of growth factors acting on themselves via functional external receptors. The autocrine action in cancer cells of growth factors such as transforming growth factor and platelet-derived growth factor has been demonstrated in certain virus-transformed cells (4–7). A number of experiments have been recently reported that show the relationship of endogenous production of a growth factor(s) to malignant transformation in fibroblasts and hematopoietic cells by means of transfection and expression of genes coding for: granulocyte-macrophage colony stimulating factor (GM-CSF),[1] transforming growth factor α, epidermal growth factor, and fibroblast growth factor (8–12).

IL-2 autocrine stimulation has been demonstrated for the in vitro growth of a human T cell line isolated from a patient with non-Hodgkin T cell lymphoma (13), and not conclusively but suggestively, a gibbon T cell line MLA144 isolated from a spontaneous lymphosarcoma (14). Furthermore, evidence has been accumulated to suggest the involvement of the aberrant activation of the IL-2 autocrine loop in the development of adult T cell leukemia (ATL) by the infection of $CD4^+$ helper T cells with human T cell leukemia virus type 1 (HTLV-1) (15–22). Recent work has shown that the infection of an IL-2-dependent murine cytotoxic T cell line (CTLL-2) with a retrovirus vector carrying the human IL-2 gene resulted in factor-independent growth in vitro and tumorigenicity in vivo (23). However, the growth autonomy of the CTLL-2 transfectants does not seem to be governed by the IL-2 autocrine mechanism as discussed later.

The generation of cells that constitutively both express IL-2-R and secrete IL-2 should provide a model system to assess the autocrine hypothesis in the malignant transformation of T cells. In the present study, we introduced an expression vector carrying a mouse IL-2 cDNA into an IL-2-dependent murine T cell line HT-2, which was derived from T cells that provide $I\text{-}A^d$-restricted help to B cells for anti-sheep red blood cell response (24). The transfectants proliferated in vitro autonomously

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[1] *Abbreviations used in this papar:* ATL, adult T cell leukemia; GM-CSF, granulocyte/macrophage colony stimulating factor; HTLV-1, human T cell leukemia virus type 1; mIL-2, mouse IL-2.

through an IL-2 autocrine mechanism and the higher producers of IL-2 developed tumors in vivo. Our results indicate that the dysregulation of an IL-2 system is involved in an autocrine fashion in the development of T cell malignancies in vivo.

Materials and Methods

Plasmid Construction. Enzymatic manipulations of DNA and preparation of plasmid DNA were performed according to standard procedure (25). A cDNA expression vector BCMGNeo was constructed from the vector BMGNeo (26) and the plasmid pRR23 (27) kindly provided to us by Dr. B. Fleckenstein, Institut für Klinische Virologie der Universtität Erlangen-Nürnberg, Erlangen, FRG. The 710-bp long Xba I-Sal I fragment containing mouse metallothionein-I promoter in BMGNeo was replaced in BCMGNeo with the 745-bp long Hind III-Sac II fragment of pRR23 carrying the human CMV promoter and enhancer after the sites of Hind III and Sac II had been converted to Xba I and Sal I sites, respectively, by ligation of synthetic linkers. BCMGNeo mIL-2 was constructed by first ligating Xho I linkers to the 563-bp long Pst I-Ssp I fragment of mouse IL-2 cDNA (a generous gift of Dr. W. Fiers, State University of Ghent, Belgium) and subsequent ligation of the fragment into the Xho I site of BCMGNeo.

Northern Blot Analysis. Preparation of total RNA, denaturation with glyoxal, electrophoresis in 1.4% agalose gel, and transfer onto nylon membrane were performed as described previously (25). The 563-bp long Pst I-Ssp I fragment of mIL-2 cDNA and the 560-bp long Xho I-Bam HI fragment containing rabbit β-globin DNA of the vector BCMGNeo were subcloned into the plasmid pSK(+) (Stratagene, La Jolla, CA). ^{32}P-labeled RNA probes complementary to mRNA were prepared from the linearized plasmids by using T3 RNA polymerase. Northern filters were hybridized overnight with ^{32}P-labeled RNA probes in 50% formamide/5 × SSC/50 mM Tris (pH 7.5)/5 mM EDTA/0.1% sodium pyrophosphate/1% SDS/0.2% polyvinylpyrolidone/0.2% ficoll/0.2% BSA/150 μg/ml denatured salmon sperm DNA at 68°C. The filters were washed at 68°C twice in 1 × SSC/0.1% SDS for 15 min each, twice in 0.3 × SSC/0.1% SDS for 15 min each, and then twice in 0.1 × SSC/0.1% SDS for 15 min each before autoradiography.

Cell Lines and Culture. An IL-2-dependent helper T cell line HT-2 derived from a BALB/c mouse (24) and the plasmid-transfected HT-2 cells were maintained in RPMI 1640 medium supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY), 100 U/ml of penicillin-streptomycin (Gibco Laboratories), 2 mM L-glutamine (Gibco Laboratories) and 5×10^{-5} M 2-ME.

DNA Transfection. DNA transfection was performed by the protoplast fusion technique. Bacteria bearing the appropriate plasmids were converted to protoplasts and fused to HT-2 cells by using polyethylene glycol 2,000 (Wako Pure Chemical Industries, Osaka, Japan) as described previously (28). 2 d after fusion cells were cultured in the presence of 1 mg/ml of G418 (Gibco Laboratories, Paisley, Scotland) and 50 U of murine (m) rIL-2 (26). Among G418-resistant transfectants, those grown in the absence of exogenous IL-2 were selected, and then subcloned by the limiting dilution method in the presence of mrIL-2. Subclones of transfectants were thereafter maintained in media without G418 and exogenous IL-2.

Assay for IL-2 Activity. IL-2 activity was measured by the [^{3}H]thymidine uptake of IL-2-dependent mouse cytotoxic T cell line CTLL-2 (29). Serial dilutions of IL-2 were incubated with 5,000 CTLL-2 cells in a volume of 200 μl for 24 h, followed by a 6-h pulse of 1 μCi of [^{3}H]thymidine (Amersham, Tokyo, Japan). 1 U of activity was defined as the amount of IL-2 that induced 50% of maximal proliferation in a 200-μl culture.

Cell Proliferation Assay. Cells were cultured at 5×10^3 cells/0.2 ml (Fig. 2 and Table II) or at indicated densities shown in Fig. 3 in the presence or absence of mrIL-2 for 24 h (Fig. 2 and Table II) or for 48 h (Fig. 3), followed by a 6-h pulse of 1 μCi of [^{3}H]thymidine, and [^{3}H]thymidine incorporation was measured. In the growth inhibition assay, rat anti-mouse IL-2-R antibodies AMT-13 (30), 7D4, and 3C7 (31); mouse anti-human IL-2 antibody DMS-1 crossreactive with mouse IL-2 molecules (32); or rat anti-mouse IL-4 antibody 11B11 (33) were added at the beginning of the cultures.

Transplantation of HT-2 Transfectants. HT-2 transfectants (10^6 or 10^7 cells) were injected sub-

cutaneously into BALB/c mice (6–8 wk old; Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan) or BALB/c *nu/nu* mice (6–7 wk old; CLEA Japan Inc., Tokyo, Japan). Mice were monitored twice a week for the appearance of solid tumor.

Results

Transfection of an IL-2-dependent Mouse Helper T Cell Line with Mouse IL-2 cDNA. An mIL-2 cDNA (cloned by W. Fiers, unpublished data), from which most of the 3′ untranslated region containing A and T nucleotide-rich sequences responsible for the destabilization of transcripts has been deleted, was inserted into the expression vector BCMGNeo derived from the vector BMGNeo (26) to construct the plasmid BCMGNeo · mIL-2. In this construct, the transcription of mIL-2 cDNA is under the control of the promoter/enhancer of human CMV (27), which has been found to be 10 times stronger than the mouse metallothionein I promoter on BMGNeo vector (our unpublished observation). A helper T cell line HT-2 derived from a BALB/c mouse (24), which is absolutely dependent on exogenously supplied IL-2 for its survival and proliferation, was transfected with BCMGNeo · mIL-2 by means of the protoplast fusion technique (34). G418-resistant stable transfectants were obtained in the presence of exogenous IL-2 at the frequency of 10^{-5}. Among these transfectants ~5% of clones could grow in the absence of added IL-2, while any of the HT-2 cells transfected with the vector BCMGNeo alone could not. The G418-resistant transfectants grown in the absence of exogenous IL-2 were subcloned and four independent clones, K9-18, K21-1, K26-16, and K28-6, were established for further analysis.

Autonomous Growth of mIL-2 cDNA-transfected HT-2 Clones through the IL-2 Autocrine Loop. To determine whether the autonomous growth of mIL-2 cDNA-transfected HT-2 clones is mediated by an IL-2 autocrine mechanism, we first examined the presence of IL-2 activity in the culture media from the HT-2 transfectants by [^{3}H]thymidine incorporation of an IL-2-dependent cytotoxic T cell line CTLL-2 (29) (Table I). The IL-2 cDNA transfectants synthesized and secreted IL-2 ranging from 20 to 400 U/10^6 cells in 48-h culture, whereas no detectable IL-2 was found

TABLE I

Secretion of IL-2 by IL-2 cDNA-transfected HT-2 Cells

Cell line	Plasmid	IL-2 secretion*
		U/ml
HT-2	–	<0.1
H6	BCMGNeo	<0.1
H15	BCMGNeo	<0.1
K9-18	BCMGNeo·mIL-2	287
K21-1	BCMGNeo·mIL-2	427
K26-16	BCMGNeo·mIL-2	76
K28-6	BCMGNeo·mIL-2	24
T6‡	BCMGNeo·mIL-2	232
EL-4§	–	1,689

* Media conditioned for 48 h by cells (10^6/ml) were tested for IL-2 activity on CTLL-2 cells by [^{3}H]thymidine uptake (see Materials and Methods).

‡ Cells of a bulk culture derived from one of tumors developed in nude mice inoculated with the IL-2 cDNA transfectant K21-1.

§ For comparison, thymic lymphoma EL-4 cells (10^6/ml) were stimulated for 48 h with 5 ng/ml of PMA to secrete IL-2.

in media conditioned by HT-2 cells untransfected or transfected with the vector alone. Northern blot analysis revealed that IL-2-specific transcripts in the transfectants carried the sequence derived from rabbit β-globin gene of the expression vector BCMGNeo (Fig. 1). Therefore, the IL-2 production of the transfectants was caused by the transfected DNA and not by the activation of an endogenous IL-2 gene.

We next examined the responsiveness to IL-2 of these IL-2-secreting HT-2 transfectants by the proliferaiton assay. As shown in Fig. 2 *A*, the transfectant K21-1 incorporated [^{3}H]thymidine in the absence of exogenous IL-2. The addition of mIL-2 (26) as well as its own conditioned media increased [^{3}H]thymidine uptake in a dose-dependent manner, indicating that the transfectant responded to IL-2 secreted by itself. K21-1 also responded to rIL-4 (26), as do untransfected HT-2 cells. A similar responsiveness to IL-2 and IL-4 was observed in the other three clones as well (data not shown). The influence of cell density on the proliferation of the transfectants was further examined, because we noticed that their growth was greatly slowed and the cell viability became lower when they were maintained at too low densities. The transfectant K21-1 maintained at relatively high density (2–5 × 10^5/ml) was replated at various cell densities ranging from 1 × 10^3 to 3.2 × 10^4 cells/ml) in the presence or absence of exogenous IL-2, and their proliferation was determined by [^{3}H]thymidine incorporation (Fig. 3). The proliferation rate expressed as [^{3}H]thymidine incorporation per cell declined as cell density became lower in the absence of exogenous IL-2 (Fig. 3 *B*). However, the addition of IL-2 rendered the rate constant regardless of cell density, as observed in untransfected HT-2 cells (Fig. 3 *A*). This result indicated that the autonomous growth of the transfectants required accumulation of autogenous IL-2 in the surrounding media. Indeed the K21-1 clone at low

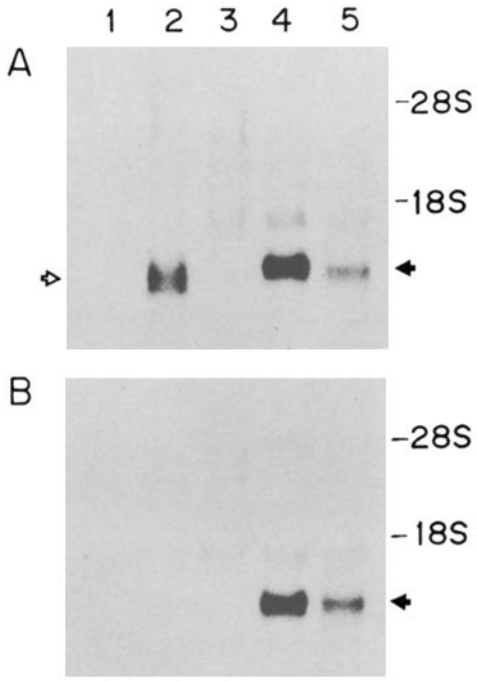

FIGURE 1. Northern blot analysis of IL-2-specific transcripts in IL-2 cDNA-transfected HT-2 cells. Total cellular RNA (20 μg) from the following cell lines was electrophoresed on 1.4% agarose gel, transferred to nylon membrane, and successively hybridized with (*A*) a ^{32}P-labeled mIL-2 cDNA probe and with (*B*) a ^{32}P-labeled rabbit β-globin gene probe derived from the vector BCMGNeo. (Lane *1*) Thymic lymphoma EL-4 cells as a control, (lane *2*) EL-4 cells stimulated with PMA, (lane *3*) untransfected HT-2 cells, (lane *4*) HT-2 transfectant K21-1, (lane *5*) HT-2 transfectant K9-18.

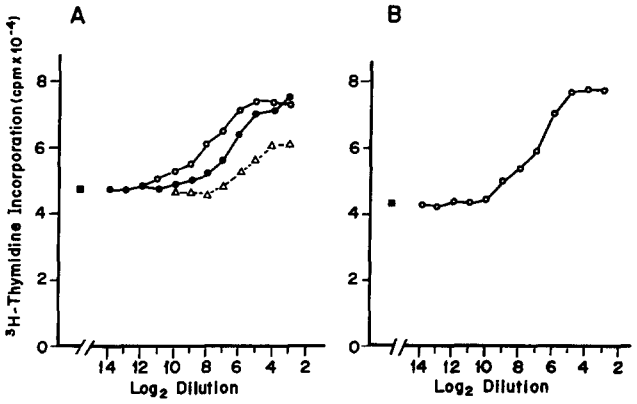

FIGURE 2. Proliferative responsiveness to IL-2 of IL-2 cDNA-transfected HT-2 cells and tumor-derived cells. (*A*) IL-2 cDNA transfectant K21-1 (5 × 10^3 cells/0.2 ml) was cultured for 24 h in the absence (■) or in the presence of serially diluted murine rIL-2 (○), murine rIL-4 (△), or media conditioned for 48 h by K21-1 cells (●), followed by a 6-h pulse of [^{3}H]thymidine. The titers of rIL-2 and rIL-4 before dilution were 640 and 40 U/ml, respectively. (*B*) Cells of a bulk culture (T6) derived from a tumor in a nude mouse inoculated with K21-1 cells were cultured in the absence (■) or presence (○) of murine rIL-2.

cell density without exogenous IL-2 could not form any colonies in soft agar (our unpublished observation).

Since all the growth properties of the HT-2 transfectants shown above suggested that their autonomous growth was operated through an IL-2 autocrine mechanism, we further examined whether reagents that block the IL-2/IL-2-R autocrine loop would affect the growth of the transfectants (Table II). The proliferation of the K21-1 clone was almost completely inhibited by mAbs against the IL-2-R: AMT-13 (30), 7D4, and 3C7 (31), which did not affect the proliferation of control HT-2 cells stimu-

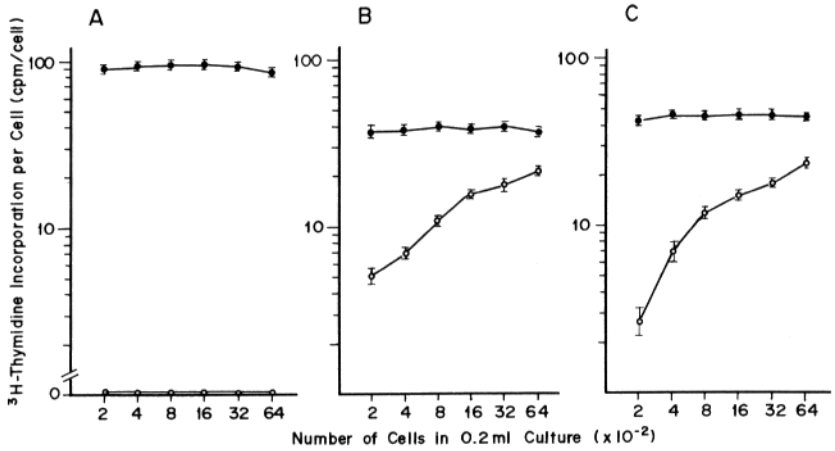

FIGURE 3. The rate of cell proliferation as a function of cell density in IL-2 cDNA-transfected HT-2 cells and tumor-derived cells. (*A*) Untransfected HT-2 cells, (*B*) IL-2 cDNA transfectant K21-1, (*C*) cells of a bulk culture (T6) derived from a tumor in a nude mouse inoculated with K21-1 cells. Cells plated at different cell densities were cultured for 48 h in the absence (○) or presence of 100 U/ml rIL-2 (●), followed by a 6-h pulse of [^{3}H]thymidine. The proliferation rate expressed as [^{3}H]thymidine incorporation (cpm) per cell at different densities is plotted on a logarithmic-logarithmic scale.

TABLE II

Inhibition of Cell Proliferation by mAbs to IL-2 and IL-2-R

mAb	Dilution	[^{3}H]thymidine uptake*		
		K21-1	T6	HT-2 stimulated with IL-4‡
			cpm	
–	–	40,168	44,395	42,010
AMT-13§	1:500	2,964 (93)‖	5,573 (87)	40,824 (3)
	1:2,500	22,906 (43)	20,407 (54)	42,667 (0)
7D4 + 3C7§	1:500	7,536 (81)	10,518 (76)	37,486 (11)
	1:2,500	24,096 (40)	22,667 (49)	42,311 (0)
DMS-1¶	1:20	8,289 (79)	21,384 (52)	39,468 (6)
	1:500	26,532 (34)	31,100 (30)	42,281 (0)
11B11¶	1:20	40,873 (0)	42,088 (5)	1,211 (97)
	1:500	38,658 (4)	41,106 (7)	7,688 (82)

* Cells (5×10^3/0.2 ml) were cultured for 24 h with or without addition of mAbs at indicated dilutions, followed by a 6-h pulse of [^{3}H]thymidine.

‡ HT-2 cells were incubated with 1.4 U of rIL-4, which induced 65% of maximal response in this condition.

§ Ascitic form.

‖ Percent inhibition is shown in parentheses.

¶ Culture supernatant of the hybridoma.

lated with IL-4. The anti-IL-2 mAb DMS-1 (32) also inhibited the proliferation of K21-1 to the same extent, whereas the anti-IL-4 mAb 11B11 (33), which completely blocked the proliferation of HT-2 cells stimulated with IL-4, had no effect on that of K21-1. These results confirmed that both IL-2 molecules secreted by the transfectants and IL-2-R expressed on their surface contributed to their autonomous growth.

Tumorigenicity of IL-2 cDNA-transfected HT-2 Clones. To assess the hypothesis that cells could become tumorigenic by autocrine growth stimulation, we tested the IL-2 cDNA-transfected HT-2 clones for their ability to cause tumors in mice. As shown in Table III, no tumors developed during an observation period of at least 20 wk in syngeneic BALB/c mice injected subcutaneously with 10^6 or 10^7 cells of the IL-2 cDNA transfectants. This was also the case in 300 rad irradiated mice. Neither intravenous nor intraperitoneal injection of cells caused tumors or leukemias in BALB/c mice. In contrast, when injected into BALB/c nude mice, three of four transfectants tested produced progressively growing tumors, while HT-2 cells untransfected or transfected with the vector alone did not (Table III: HT-2, H6, H15). Two clones, K9-18 and K21-1, which secreted 287 and 427 U/ml of IL-2, respectively, were tumorgenic when 10^6 or 10^7 cells were inoculated. Two of five nude mice injected with 10^7 cells of K26-16, the IL-2 production of which was 76 U/ml, developed tumors, while the inoculation of 10^6 cells did not produce any tumors. On the other hand, K28-6, the lowest producer of IL-2 (24 U/ml), was not tumorigenic even when 10^7 cells were injected. Among the tumorigenic clones the latency period of tumor development varied from 29 to 75 d (Table III).

To address the question as to whether these differences among clones in tumorigenicity and the latency period would be based on an unknown property specific to

TABLE III

Tumorigenicity of IL-2 cDNA-transfected HT-2 cells

Cell line	BALB/c		BALB/c (300 rad irradiated)		BALB/c *nu/nu*	
	10^6 cells	10^7 cells	10^6 cells	10^7 cells	10^6 cells	10^7 cells
HT-2	0/5	0/5	0/5	0/5	0/5	0/5
H6	0/5	0/5	0/5	0/5	0/5	0/5
H15	0/5	0/5	0/5	0/5	0/5	0/5
K9-18	0/5	0/5	0/5	0/5	5/5 (64 d)*	5/5 (49 d)
K21-1	0/5	0/5	0/5	0/5	8/8 (39 d)	10/10 (29 d)
K26-16	0/5	0/5	0/5	0/5	0/5	2/5 (75 d)
K28-6	0/5	0/5	0/5	0/5	0/5	0/5

BALB/c (either irradiated or not) and BALB/c *nu/nu* mice were inoculated subcutaneously with the indicated number of cells from individual cell lines (see Table I for the plasmids introduced into cell lines). The animals were observed for a minimum period of 20 wk for the formation of tumors >1 cm in diameter. Results are expressed as the fraction number of mice with tumor per number of mice injected.

* The average of time after inoculation before tumors became visible.

each clone or would just correlate with the level of IL-2 production, 10 subclones derived from K21-1, which differed in the level of IL-2 secretion (120–1,227 U/ml), were tested for their tumorigenicity in nude mice (Fig. 4). Although all of the subclones (10^7 cells per injection) produced tumors by 70 d after injection, the latency period of tumor development showed a clear correlation with the level of IL-2 secreted by an individual subclone: a higher producer of IL-2 gave rise to a tumor in a shorter period.

We next examined the growth properties in vitro of tumor cells to test the possibility that a mutation(s) in vivo such as an activation of an oncogene(s) conferred autonomous growth on cells through a mechanism(s) other than IL-2 autocrine self-stimulation. First, cells from tumors developed in nude mice were all G418 resistant, indicating that tumors were derived from the transfectants inoculated. Second, cells from tumors, T6 for example, retained the ability of IL-2 production and the proliferative responsiveness to IL-2 (Table I and Fig. 2 *B*). Third, the proliferation in vitro of tumor cells was a function of cell density in the absence of exogenous IL-2 (Fig. 3 *C*) and was blocked by antibodies to IL-2 or IL-2-R (Table II). Thus, the growth properties in vitro of tumor cells were indistinguishable from those of injected transfectants.

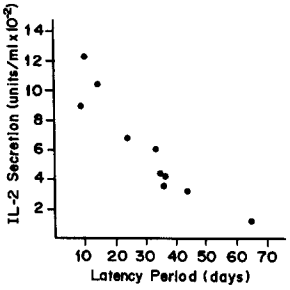

FIGURE 4. Close relationship between the latency period of tumor development and the level of IL-2 secretion. IL-2 cDNA transfectant K21-1 was subcloned by limiting dilution method, and 10 subclones, which differed in the level of IL-2 secretion, were chosen for analysis of their tumorigenicity. Four nude mice were inoculated subcutaneously with 10^7 cells of each subclone. The average of time (days) after inoculation before tumors become >1 cm in diameter is plotted vs. the level of IL-2 secreted in vitro by each subclone (units/milliliter/10^6 cells/48 h).

Discussion

The autocrine nature of the IL-2 cDNA-transfected HT-2 cells established in this experiment was warranted by four lines of evidence: (*a*) the transfectants constitutively secreted biologically active IL-2 into the culture media; (*b*) they responded by proliferation to added rIL-2 as well as to their own secreted IL-2; (*c*) their growth was a function of the cell density; and (*d*) the proliferation was inhibited by mAbs to IL-2 as well as to IL-2-R. The data are consistent with the concept of autocrine self-stimulation but not with the growth autonomy by spontaneous mutations.

Recent work by Yamada et al. (23) has demonstrated that a murine cytotoxic T cell line CTLL-2 infected with a retroviral expression vector carrying a human IL-2 cDNA proliferated in vitro in the absence of exogenous IL-2 and developed tumors when injected into syngeneic or nude mice. However, the growth property of the CTLL-2 transfectants is different from that of our HT-2 transfectants. First, the secretion of IL-2 from the CTLL-2 transfectants was hardly detectable in the culture supernatant. One may argue that this is due to the continuous consumption of IL-2 for their autonomous growth. However, that is not the case in our HT-2 transfectants, because the accumulation of autogenous IL-2 in the culture media is required for the autonomous growth of the HT-2 transfectants (Fig. 3). Indeed, when the HT-2 transfectants were cultured at too low cell density, IL-2 activity was undetectable in the culture supernatants, and the transfectants died. Second, the CTLL-2 transfectants did not have responsiveness to exogenous IL-2. The addition of IL-2 did not augment but rather suppressed their proliferation. The effect of anti-IL-2 antibodies to the proliferation was not examined in their study. Third, an anti-IL-2-R mAb, AMT-13, blocked only partially the proliferation of the CTLL-2 transfectants even at a higher concentration than that sufficient to inhibit completely the IL-2-induced proliferation of untransfected CTLL-2 cells. Thus, the growth autonomy observed in the CTLL-2 transfectants seems to be mediated through a different mechanism than that in the HT-2 transfectants. It is possible that in the CTLL-2 transfectants the ligand may interact with the receptor mainly at some intracellular location, being distinct from the external receptor–ligand interaction actually occurring in our HT-2 transfectants. However, no experimental proof is available at the moment.

The establishment of T cell clones that proliferated autonomously through autocrine mechanism gave us the opportunity to assess the autocrine hypothesis that states: cells could escape from the normal growth control and become malignant by endogenous production of a growth factor(s) acting on themselves. Supporting this hypothesis is the presence of a human T cell lymphoma line that secretes IL-2 and expresses IL-2-R (13). mAbs directed against either IL-2 or IL-2-R blocked the proliferation of this malignant T cell line. In the present study we showed that the T cell clones proliferating in vitro by IL-2 autocrine self-stimulation developed tumors when injected into nude mice (Table III). The tumorigenicity apparently correlated with the level of IL-2 secreted. A clone expressing a low level of IL-2 did not produce any tumors, in contrast to the CTLL-2 transfectants described by Yamada et al. (23). Among tumorigenic clones of the HT-2 transfectants, the latency period of tumor development showed the clear relationship to the level of IL-2 secreted by an individual clone (Fig. 4). Interestingly, none of the HT-2 transfectants, regardless of the level of IL-2 secretion, developed tumors in syngeneic BALB/c mice, unlike the CTLL-2 transfectants.

It is unlikely that the HT-2 transfectants could become tumorigenic in vivo by a mutation(s) that abrogated the requirement for IL-2 autocrine self-stimulation, because cells obtained from tumors proliferated in vitro in an IL-2 autocrine fashion indistinguishable from that of the inoculated cells (Tables I and II, Figs. 2 *B* and 3 *C*). It has been known that bovine papilloma virus DNA present in the expression vector BCMGNeo can transform certain epithelial and fibroblastic cell lines such as NIH3T3, FR3T3, and C127 cells (35, 36), but not other types of cells. In fact, none of HT-2 cells transfected with the vector alone produced tumors in vivo (Table III: H6, H15). Thus, it is likely that an aberrant operation of the IL-2 autocrine mechanism leads T cells to malignant transformation. It remains to be clarified whether the IL-2 autocrine self-stimulation in itself is sufficient for tumorigenesis or whether other synergistic event(s) are required to occur concomitantly.

It has been suggested that the aberrant activation of an IL-2 autocrine loop in $CD4^+$ helper T cells infected with HTLV-1 contributes to the pathogenesis of ATL. ATL cells as well as T cells transformed by HTLV-1, unlike normal activated T cells, constitutively express IL-2 receptors on their cell surfaces (15, 17, 37), suggesting the abnormal regulation of IL-2-R expression in those cells. While HTLV-1 does not contain a viral oncogene related to a cellular gene (38), it possesses a trans-activator (*tat I* or $p40^x$) gene whose product is able to stimulate transcription of other viral genes from the viral long terminal repeat (39–42). Tat I has been revealed to activate the expression of genes encoding IL-2-R as well as IL-2 in T cells (19–22). Some in vitro cell lines established from ATL patients have been reported to constitutively secrete and respond to IL-2 (16). It has also been demonstrated that leukemic cells in the peripheral blood of a patient with ATL responded to IL-2 secreted by themselves and their proliferation was inhibited by anti-IL-2 or anti-IL-2-R mAbs (18). The resemblance with the growth property of these ATL cells and our HT-2 transfectants is striking. It is likely from our results that a higher producer of IL-2 has a growth advantage and thereby becomes a predominant clone among HTLV-1-infected T cells that proliferate polyclonally by IL-2 autocrine self stimulation.

In most cases of ATL, with some exceptions (43–44), leukemic T cells respond poorly to IL-2 in vitro (45) and rarely produce IL-2 (46), which suggests an additional abnormality(ies) rendering infected T cells to go from IL-2 dependent to IL-2 independent. We obtained several variant clones from mIL-2 cDNA-transfected HT-2 cells after a long-term in vitro culture (unpublished experiments). While these variants retained the ability to secrete IL-2, they did not respond to exogenous IL-2 and their proliferation was not inhibited any more by antibodies to IL-2 or IL-2-R. Such a transition from a factor-dependent to -independent state has been also observed in GM-CSF-dependent cell lines infected with a retrovirus vector carrying GM-CSF cDNA, though their tumorigenicity has not been tested (47). These observations may indicate what would happen in HTLV-1-infected T cells. Our proposition in this paper is that the IL-2 autocrine self-stimulation is involved in at least some stage(s) of the transforming process of HTLV-1-infected T cells. In efforts to further elucidate the role of IL-2 autocrine mechanism in the development of ATL and other T cell malignancies, it will be of interest to determine how an additional genetic alteration(s) correlates with IL-2 autocrine self-stimulation in the variant clones of HT-2 transfectants.

Summary

We introduced a mouse IL-2 cDNA expression vector into an IL-2-dependent mouse helper T cell line HT-2. Transfected cells secreted substantial amounts of IL-2, to which they themselves responded by proliferating without further requirement for exogenous IL-2. The proliferation was a direct function of the cell density and was inhibitable by antibodies against IL-2 or IL-2-R, indicating the autocrine nature of the proliferation. Those producing higher amounts of IL-2 were found to be tumorigenic when inoculated into nude mice. The latency period of tumor development correlated inversely with the level of IL-2 secreted. Tumor cells proliferated in vitro in an IL-2 autocrine fashion indistinguishable from that of the inoculated cells. We thus provide evidence that the aberrant activation of the IL-2 autocrine circuit can lead T cells to malignant transformation.

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