Lack of Interleukin-2 (IL-2) Dependent Growth of TAC Positive T-ALL/NHL Cells is Due to the Expression of Only Low Affinity Receptors for IL-2

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Binding of interleukin-2 (IL-2) to high affinity receptors on activated normal T cells was shown to be the essential step in induction of proliferation of such cells. The finding of abundant IL-2 receptors on malignant T cells in adult T cell leukemia suggested a deregulation of the IL-2/IL-2 receptor system and was assumed to account for aberrant growth in malignant disorders of T cells. In this study we use malignant T cells from nine patients with the clinical diagnosis of T-ALL or T-NHL and did not detect IL-2 dependent growth under conditions in which normal T cells responded to IL-2. IL-2 receptors comparable in numbers to activated T cells were found on T-ALL/T-NHL cells stimulated with PHA and PMA. However, binding studies using radiolabeled IL-2 indicated that the receptors present on malignant T cells were not able to bind to IL-2 with high affinity. Therefore, if IL-2 is involved in the proliferation of malignant T cells, its mechanism of growth regulation may be different from the one for normal T cells. Alternatively, IL-2 may not play a role in the regulation of growth of malignant T cells in vitro.

INTRODUCTION

AFTER activation by antigen or mitogen, T cells secrete interleukin-2 (IL-2), express IL-2 receptors, and subsequently start to proliferate (1–3). Thus, proliferation of activated clones is mainly dependent on a single growth factor (IL-2) and its membrane receptor. The expression of IL-2 receptors on activated T cells is detected by monoclonal antibodies against epitopes of the IL-2 receptor molecule (4–6). Recently, it has been shown that the IL-2 receptor consists of at least two chains with molecular weights of 55 kd (Tac protein) and 75 kd, respectively (7–11). Only the p55 chain is recognized by anti-Tac antibodies initially used to define the IL-2 receptor (6). However, proliferation of activated T cells requires high affinity binding of IL-2 which only takes place if both chains of the receptor are expressed (9, 10).

The IL-2 dependent growth control of mature normal T cells suggests a role of IL-2 also for abnormal proliferation in T cell leukemias and non-Hodgkin's lymphomas (NHL) of T cell type. In these cases rapid proliferation of malignant cells leads to considerable tumor masses in bone marrow, the thymus, and lymphatic organs. It was originally postulated that a deregulation of the IL-2/IL-2 receptor system was involved in the proliferation of adult T cell leukemia/lymphoma (ATL) induced by HTLV I (12, 13). In fact, using the anti-Tac antibody, ATL cells have been found to express about 10 times more IL-2 receptors than stimulated T cells (14, 15). However, so far only in a few cases of ATL IL-2 dependent growth of malignant cells have been demonstrated (16). On the other hand, in a semisolid agar culture system, IL-2-mediated proliferative response of T-ALL/NHL cells has been demonstrated (17).

We compared IL-2 receptor expression and IL-2 responsiveness of T-ALL/T-NHL cells with that of normal T cells. Our data show that the malignant T cells could easily be induced to express IL-2 receptors detected by monoclonal antibodies after stimulation with phytohemagglutinin (PHA) and phorbolmyristate-acetate (PMA). However, in contrast to normal T cells, significant IL-2 dependent growth or increase in cell numbers did not occur in a standard liquid culture system. Further studies showed that the IL-2 receptors present on stimulated malignant T cells did not bind to IL-2 with high affinity.

MATERIALS AND METHODS

Patient Data and Isolation of Malignant T Cells. A summary of the patient data including phenotyping of the malignant cells is shown in Table 1. Malignant cells were isolated from the different sources indicated by standard Ficoll-Hypaque (Pharmacia, Freiburg, F.R.G.) centrifugation and stored frozen in liquid nitrogen in medium containing 20% FCS and 10% DMSO. Only samples containing more than 95% blast cells, based on morphologic examination and phenotyping, were included in the study. The phenotypes of freshly isolated and thawed cells were identical. The viability of thawed cells was greater than 95%. All patients were included in the German BFM ALL/NHL therapeutic protocols, and phenotyping of T-ALL/NHL was confirmed by the study's reference typing laboratory (Divison of Hematology, Klinikum Steglitz, West Berlin, F.R.G.).

ALL patients exhibited the typical clinical picture of T-ALL/NHL with enlarged lymph nodes and mediastinal mass. Discrimination between ALL and NHL was made on the basis of bone marrow involvement.

Isolation of T Cells from Donor Blood. T cells were isolated from the mononuclear cell fraction of peripheral blood after Ficoll-Hypaque centrifugation by rosetting with sheep erythrocytes treated with 2-aminethylthiouroniumbromide (AET) as described (18). Briefly, cells were incubated with AET treated sheep erythrocytes, and rosettes were isolated by Ficoll-Hypaque centrifugation. Erythrocytes in the sedimented fraction were removed by hypotonic lysis in distilled water for 5 sec followed by reconstitution to normal osmolarity using excess culture medium.

Monoclonal Antibodies (MoAb) and Immunofluorescence. MoAbs reactive with T11, T3, T4, T8 antigen were purchased from Ortho Diagnostics (Neckargemünd, F.R.G.) and used according to the manufacturer's instruction. The pan-T antibody Leu-1 and Leu-9, an antibody reactive with all T-ALL cells, corresponding to the WT1 antibody (19, 20), were purchased from Becton-Dickinson (Heidelberg, F.R.G.). Control antibodies not listed in Table 1 included anti-HLA-DR (Becton-Dickinson), anti-CALLA (J5, Coulter Electronics, Krefeld, F.R.G.), and the B cell specific antibodies HD 37 (CD19) and HD 39 (CD22) provided by Dr. B. Dörken (Medizinische Universitäts-Poliklinik, Heidelberg). The MoAb against the IL-2 receptor (anti-Tac) was kindly

Received January 23, 1989. Accepted March 2, 1989.

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0887-6924/89/0308-0566$2.00/0
LEUKEMIA

Table 1. Summary of Clinical Data and Surface Phenotype of Cells from ALL/NHL Patients

Patient	No.	Age (yr)	Sex	% Positive Cells[a]							Classification of ALL/NHL[c] (Group)	Cell Source[d]
				Leu-9	Leu-1	T11	T4	T8	T3	IL-2rec.[b]		
Z.M.	1	8	Male	95	98	3	1	1	2	0	I (NHL)	LNC
H.D.	2	8	Male	96	21	4	1	3	3	1	I (ALL)	PBL
M.C.	3	13	Male	90	25	19	1	8	1	1	I (ALL)	BM
B.C.	4	7	Male	56	72	56	4	7	12	0	I (ALL)	PBL
M.F.	5	5	Male	88	76	94	25	10	4	1	II (NHL)	PE
B.M.	6	3½	Female	66	65	95	56	93	5	1	II (ALL)	BM
L.B.	7	7½	Male	96	88	93	90	56	4	1	II (NHL)	PE
L.O.	8	10	Male	68	65	79	35	68	62	1	III (NHL)	PE
Z.M.	9	13	Male	93	92	53	14	95	71	1	III (ALL)	BM
Control T cells (AET-E$^+$)[e]				73	68	86	62	23	71	3		

[a]The phenotyping of leukemic cells was performed as described in Material and Methods.
[b]The presence of IL-2 receptors (anti-Tac) was determined on freshly isolated, unstimulated cells.
[c]The differentiation between ALL and NHL was made on the basis of bone marrow involvement (>25% blast cells).
[d]Cell sources were: LNC, lymph node cells; PBL, peripheral blood cells; BM, bone marrow cells; PE, pleural effusion cells. All cell populations studied contained more than 95% blast cells.
[e]Control T cells were prepared by rosetting with AET-treated sheep red blood cells as indicated in Material and Methods; the purity of isolated T cell population always exceeded 95%.

provided by Dr. T. Waldmann, National Cancer Institute, and was used in 1:20 dilution of the purified antibody preparation.

Surface markers were assayed by indirect immunofluorescence using affinity purified FITC labeled goat anti-mouse IgG after incubation of 1×10^6 cells/ml with the respective MoAbs. Stained cells were either identified by immunofluorescence microscopy (Zeiss, F.R.G.) or by FACS analysis (Ortho Diagnostics).

Cell Culture and Proliferation Assay. The standard culture medium for all assays was RPMI 1640 (Gibco, Grand Island, NY) supplemented with L-glutamine (2 mM final concentration), streptomycin (100 μg/ml), penicillin (100 U/ml), HEPES buffer (25 mM final concentration), and 10% fetal calf serum (FCS) (Cat. No. 20017, Conco Lab-Division, Wiesbaden, F.R.G.). All cell cultures were performed at 37°C in air with 5% CO_2 and at 90% relative humidity.

For the assessment of proliferative responses, cells were cultured for 3 days in 96-well flat bottom plates (Costar, Darmstadt, F.R.G.) in the presence of irradiated (3,000 rad) peripheral blood mononuclear cells (1×10^5/well) as a feeder layer. Stimulation included 1 μg/ml PHA (PHA-P, Wellcome, Burgwedel, F.R.G.) and 10 ng/ml PMA (12-o-tetradecanoylphorbol-13-acetate, Sigma, Munich, F.R.G.). Recombinant IL-2 (r-IL-2 100 U/ml), kindly provided by Biogen S.A., Geneva, was added to some cultures. For the last 8 hr of culture 0.5 uCi ^{3}H-thymidine (^{3}H-TdR, specific activity 25 Ci/mM, Amersham, Braunschweig, F.R.G.) was added. Incorporation of ^{3}H-TdR was measured using standard liquid scintillation procedures.

Induction of IL-2 Receptor Expression. 1×10^6 cells/ml were cultured for 48 hr in the presence of 1 μg/ml PHA and 10 ng/ml PMA, washed twice, and assayed for IL-2 receptor expression as indicated.

IL-2 Dependent Growth of Activated Cells. After activation by PHA and PMA, as described in the last paragraph, cells (1×10^5/well) were cultured in 96-well flat bottom plates for 24 hr in the presence of IL-2 (100 U/ml) and 0.5 uCi ^{3}H-TdR to assess proliferation.

In addition, activated cells were cultured in a concentration of 1×10^5/ml in 24-well plates (Costar, Darmstadt, F.R.G.) in the presence of r-IL-2 (100 U/ml) for 1 week. On day 4 additional IL-2 (100 U/ml) was added to the cultures. The proliferative response was assessed by counting viable cells (trypan blue exclusion) on day 4 and day 7.

^{125}J-IL-2 Binding Assay. Normal and malignant T cells were stimulated for 48 hr with PHA and PMA as described before. After extensive washing in RPMI 1640 without FCS, cells were incubated two times at 37°C for 1 hr in culture medium without FCS and washed again. For IL-2 binding, 80 μl containing 1×10^6 cells in RPMI 1640 + 1% bovine serum albumin (BSA) were incubated with 10 μl ^{125}J-labeled IL-2 (NEN, Boston, MA) in 1.5-ml Eppendorf tubes to give final concentrations ranging from 1 pM to 500 pM ^{125}J-IL-2. In order to determine specific binding, parallel tubes included a 100-fold excess of cold r-IL-2. Incubation was done at 40°C for 1 hr. After incubation, cells were centrifuged at 10,000 × *g* for 30 sec, resuspended, and centrifuged through a 200 μl layer of a mixture of 77% dibutylphthalate and 23% olive oil for 90 sec at 10,000 × *g*. The tips of the tubes containing the cell pellet were cut off, and the radioactivity was measured in a gamma counter. Specific binding was calculated by subtracting activity bound in the presence of excess cold IL-2 (100-fold) from total binding.

RESULTS

Clinical Data and Phenotype of T-ALL/NHL Cells. Table 1 summarizes the clinical data and the surface phenotype of malignant cells from the patients included in this study. The MoAbs used for phenotyping had been selected because they define distinct stages of T-cell differentiation (see ref. 19). The pan T antibodies Leu-1 (CD5) and Leu-9 (CD7) were used as the most sensitive markers for T-ALL (20). The classification of T-ALL/NHL cells was made according to a recently published proposal (19). In group I the antigens found on mature T cells (T3, T4, T8) are not expressed. Group II is defined by coexpression of T4 and T8 antigens and limited expression of the T3 complex representing the antigen recognition complex of mature T cells. In group III segregation of T4 and T8 antigens occurs, and T3 is fully expressed. In all samples used for this study, more than 95% of the cells were blasts on the basis of morphologic examination. In cases 4, 8, and 9, a low contamination by normal T cells could not be excluded by phenotyping. However, stimulation by conventional T-cell mitogens (Table 2 and data not shown) did not induce proliferation in these cells.

Proliferative Response of Stimulated T-ALL/NHL Cells. In order to study the proliferation of malignant T cells induced by a T cell mitogen, normal T cells and ALL/NHL cells were stimulated with PHA for 72 hr. To reduce possible limiting factors during the culture, an excess of IL-2 (100 U/ml) and irradiated mononuclear cells as a source of other cytokines were added. As shown in Table 2, in contrast to normal T cells, no significant proliferative response could be induced in T-ALL/NHL cells.

Segregation of IL-2 Receptor Expression and IL-2 Responsiveness of Malignant T Cells. We next addressed the question as to whether IL-2 receptors are present on stimulated malignant T cells. For normal T cells, stimulation with the combination of PHA and PMA usually induces maximal IL-2 receptor expression. Our initial experiments have shown that this combination is also the most effective for induction of Tac expres-

Table 2. PHA Stimulation and IL-2 Response of T-ALL/NHL Cells (72-Hr Culture)

Patient No.	Cells Incubated with	
	Medium	PHA + IL-2
1	ND	ND
2	120	530
3	2,070	3,180
4	5,830	5,270
5	4,590	4,880
6	1,250	2,290
7	610	600
8	3,110	4,910
9	1,610	3,030
Control T cells	1,320	32,420

1×10^5 cells were incubated in flat bottom microtiter plates in the presence of feeder cells (1×10^5/well) or in the presence of feeder cells plus 1 μg/ml PHA and 100 U/ml r-IL-2 for 72 hr. Proliferation was measured after an 8-hr pulse with ^{3}H-TdR before harvesting. Data are expressed as the mean cpm of triplicate cultures with a SD of less than 10%. ND = not done.

sion on T-ALL/NHL cells. After 48 hr of incubation, Tac expression is optimal, and further incubation leads to a significant increase in the number of dead cells in the malignant cell population (Table 3). As shown in Table 1, freshly isolated unstimulated malignant cells do not express IL-2 receptors. However, with one exception (patient 5), after stimulation with PHA and PMA, a significant number of IL-2 receptor positive cells were found, using the anti-Tac MoAb (Table 4). Surprisingly, in a short term assay these IL-2 receptor positive cells did not show a significant proliferative response comparable to normal T cells (Table 5) when cultured in the presence of 100 U/ml r-IL-2. The maximal response was 9-fold (patient 9) in contrast to the 250-fold response observed with normal T cells.

Three of the malignant T cell populations (nos. 1, 3, and 9) showed IL-2 receptor expression comparable to normal T cells with respect both to the number of positive cells and fluorescence intensity of individual cells (Table 4 and Fig. 1). However, in a 1-week liquid culture system, activated cells from these patients died in the presence of IL-2 (100 U/ml), whereas activated normal T cells showed a 20–30-fold increase in cell numbers (Fig. 2). The number of viable cells and dead cells together in the cultures of the malignant cells did not exceed the initial number of cells placed in culture, indicating that no exhaustive proliferation with a high number of dead cells occurred.

^{125}J-IL-2 Binding to Stimulated Normal and Malignant T Cells. The data shown before suggested that the IL-2 receptor expression on malignant T cells defined by the anti-Tac antibody was distinct from that on normal T cells. Therefore, IL-2 binding assays were performed using the stimulated cells from the patients with the highest Tac expression (nos. 1, 3, and 9).

Table 3. Kinetic of PHA + PMA Induced IL-2 Receptor Expression

Patient No.	% Tac-Positive cells (% Viable Cells)		
	24 Hr	48 Hr	72 Hr
1	70 (95)	84 (91)	80 (48)
3	32 (97)	80 (95)	78 (54)
T cells	60 (92)	70 (95)	75 (87)

Normal and malignant T cells were incubated (1×10^6/ml) for the times indicated (hrs) in the presence of PHA (1 μg/ml) and PMA (10 ng/ml). The IL-2 receptor positive cells were detected by indirect immunofluorescence with the anti-Tac antibody and 200 cells were counted. Viability was simulateously assessed by trypan blue exclusion.

Table 4. Induction of IL-2 Receptor Expression of T-ALL/NHL Cells by PHA + PMA (48 Hr)

Patient No.	% Positive Cells	
	Unstimulated	Stimulated
1	1	87
2	4	18
3	1	89
4	1	40
5	1	8
6	5	28
7	4	24
8	6	12
9	1	45
Control T cells	5	57

Normal and malignant T cells were incubated (1×10^6/ml) for 48 hr in the presence of PHA (1 μg/ml) and PMA (10 ng/ml). The IL-2 receptor positive cells were detected by indirect immunofluorescence with the anti-Tac antibody, and 200 cells were counted.

As shown in Fig. 3, high affinity binding of ^{125}J-IL-2 was found in normal T cells stimulated with PHA plus PMA in the range of concentrations expected from the literature (1, 22, 23) with about 5,000–7,000 high affinity binding sites per cell. In contrast, the stimulated malignant T cells did not exhibit significant high affinity binding sites.

In preliminary experiments using IL-2 concentrations in the nM range, low affinity binding was observed (data not shown). Thus, the presence of the Tac antigen and the absence of high affinity binding sites suggest that only the p55 chain of the IL-2 receptor is expressed by the malignant T cells.

DISCUSSION

The proliferation of mature T cells activated by antigen or mitogen depends mainly on the presence of IL-2 as a growth factor and the expression of IL-2 receptors on the responding cell population. When IL-2 receptors are continuously expressed, T cells can be kept in culture growing for weeks or even months. The availability of recombinant IL-2, as well as monoclonal antibodies recognizing the IL-2 receptor, has allowed detailed studies of ligand receptor interaction (22–24). IL-2 was demonstrated to be bound by high affinity receptors ($kd = 10^{-11}$ M) on activated T cells (1, 23). Monoclonal antibodies such as anti-Tac which block IL-2 binding appear to recognize a 55-kd peptide as part of the IL-2 receptor (25–28). However, the number of Tac binding sites on activated T cells was always found to exceed the number of IL-2 high affinity binding sites (22, 23). Using a 1,000-fold increase of the IL-2 concentration, binding of IL-2 to the receptors defined by anti-Tac could be demonstrated. This led to the definition of Tac as the low affinity receptor and the combination of Tac

Table 5. Proliferation of Activated Normal and Malignant T Cells in the Presence of IL-2

Patient No.	Treatment of Cells	
	Unstimulated (cpm)	Stimulated (cpm)
2	296	1,357
5	671	1,845
6	564	4,817
7	1,221	6,293
9	322	2,840
Control T cells	199	49,418

Normal and malignant T cells activated by PHA (1 μg/ml) and PMA (10 ng/ml) for 48 hr were cultured (1×10^5/well) in the presence of IL-2 (100 U/ml) and ^{3}H-TdR for 24 hr. Data are given as mean cpm of triplicate cultures with a SD of less than 10%.

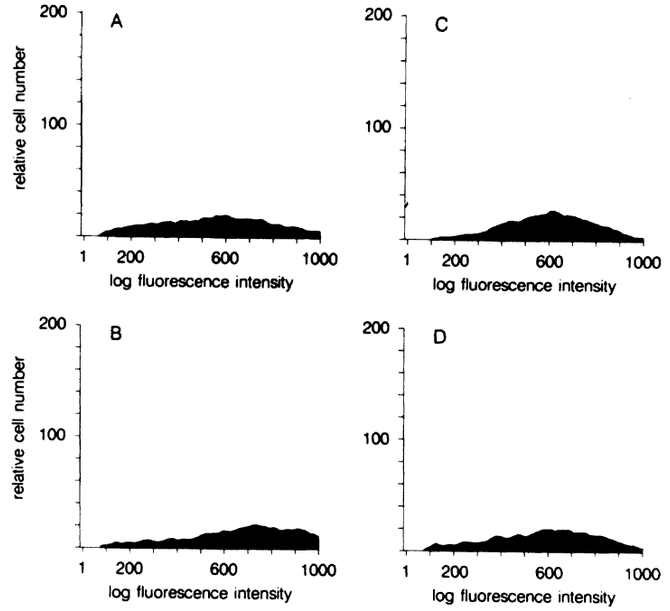

Figure 1. Comparison of Tac expression of stimulated normal and malignant T cells. Normal T cells (A) and malignant T cells (1 × 10^6/ml) from patient no. 1 (B), no. 3 (C), and no. 9 (D) were incubated for 48 hr with PHA (1 μg/ml) and PMA (10 ng/ml). Subsequently, IL-2 receptor expression was assessed by indirect immunofluorescence with the anti-Tac antibody and analyzed by flow cytometry (FACS). Data are given as fluorescence intensity versus relative cell numbers.

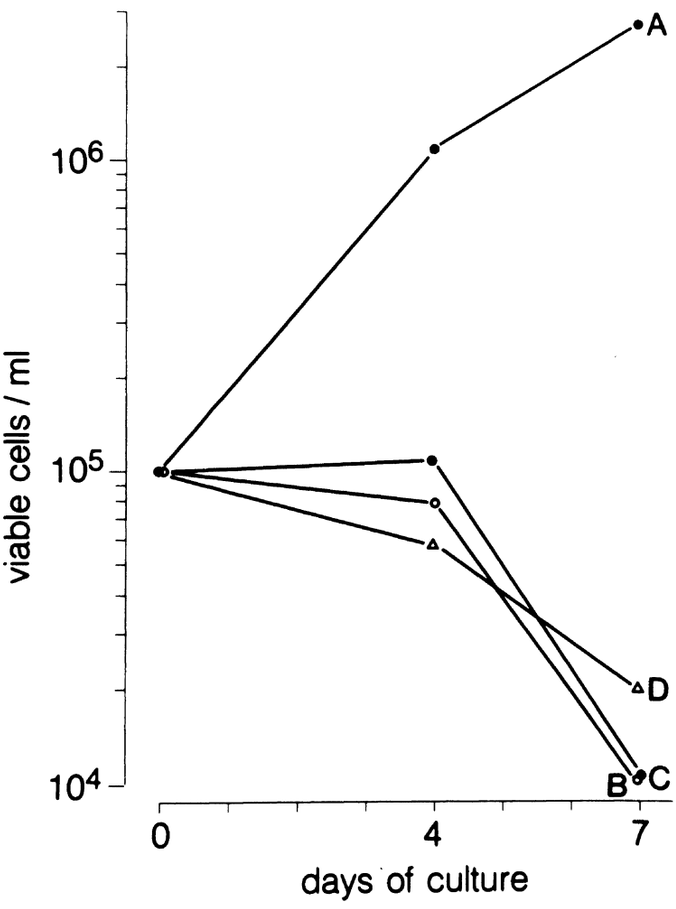

Figure 2. IL-2 induced proliferation of stimulated normal and malignant T cells. PHA + PMA stimulated cells prepared as described in Figure 1 for immunofluorescence analysis were cultured (1 × 10^5/ml) in the presence of r-IL-2 (100 U/ml). On day 4 cells were counted and r-IL-2 (100 U/ml) was again added to the cultures. Proliferation was assessed by counting viable cells on the days indicated. A, T cells; B, patient 1; C, patient 3; D, patient 9.

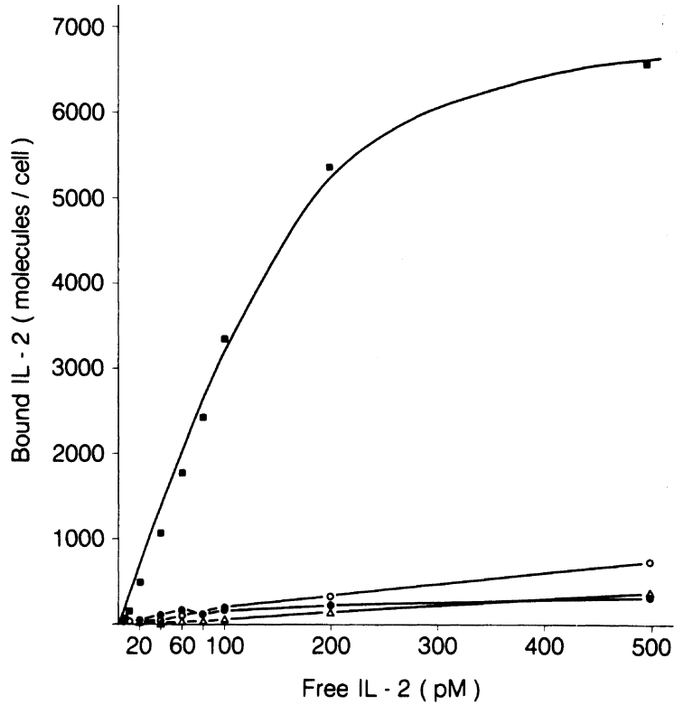

Figure 3. Binding of radiolabeled IL-2 to stimulated normal (■) and malignant T cells. Normal (■) and malignant T cells from patients 1 (○), 3 (△), and 9 (●) were stimulated to express IL-2 receptors by incubation with PHA and PMA as described (Fig. 1). Subsequently, stimulated cells were tested for IL-2 receptor expression by immunofluorescence and used for binding of ^{125}J-IL-2 as described in Material and Methods. Number of Tac positive cells in this experiment: T cells, 65%; no. 1, 85%; no. 3, 61%; no. 9, 63%.

and a postulated second component as the high affinity IL-2 receptor (22, 23).

Recently, the second component of the IL-2 receptor (7, 9, 10) could be identifed as a 75-kd protein which together with the 55-kd peptide combines to give a fully active high affinity receptor (8, 10). Only the coexpression of the 55-kd and 75-kd peptides appears to mediate IL-2 induced proliferation in mature T cells.

It was attractive to consider a deregulated IL-2/IL-2 receptor system as the cause of abnormal growth regulation in malignant T cells. In particular, continuous expression of IL-2 receptors, autocrine mechanisms assuming simultaneous production of IL-2 and IL-2 receptor expression, have been discussed (12, 13, 29). This hypothesis has been stimulated mainly by the demonstration of excess numbers of IL-2 receptors on leukemic T cells in ATL induced by human T leukemia virus type I (HTLV I) (12, 13, 30). However, freshly isolated cells express mainly low affinity receptors, do not secrete IL-2, and poorly respond to exogenous IL-2 (31, 32). On the other hand, infection of T cells by HTLV-I leads to disturbance of the IL-2/IL-2 receptor system, which may result in IL-2 dependent autonomous growth (33, 34). In addition, IL-2 dependent growth of malignant cells has been described in rare cases of ATL (16, 35). In contrast to ATL, where cells taken from patients can be maintained in vitro, the malignant T cells in T-ALL or T-NHL not induced by HTLV-I rapidly die in vitro despite extensive proliferation in vivo.

This situation strongly suggests a lack of growth factor(s) during the in vitro culture. Recently, Touw et al. demonstrated induction of IL-2 receptors and IL-2 dependent colony for-

mation in a semisolid agar culture system with malignant cells from children with T-ALL or T-NHL (17).

In our study of malignant T cells from nine patients with T-ALL or T-NHL of various differentiation stages, we found expression of IL-2 receptors as defined by the presence of anti-Tac similar to normal T cells after stimulation with PHA and PMA (Table 3). The number of receptor positive cells in two cases with early T cell phenotype (nos. 1 and 3) even exceeded that usually found with stimulated normal T cells. However, compared to normal T cells, no net gain in viable cells in vitro was induced by the addition of excess r-IL-2 to these strongly IL-2 receptor positive T-ALL/NHL cells (Fig. 2). The minimal responses observed in the short term culture system could be due to a very low fraction of responding cells or to contamination with normal T cells. If one considers the minimal ^{3}H-TdR uptake in Table 5 as indicative for a low fraction of responding cells, IL-2 is apparently not able to induce long term growth (Fig. 2) in the vast majority of IL-2 receptor positive cells. The production of growth inhibiting factors by stimulated leukemia cells has to be considered; however, supernatants of the T-ALL/NHL cells stimulated with PHA and PMA did not suppress the proliferation of IL-2 dependent T cell lines or mitogen activated T cells (data not shown).

Our binding studies using radiolabeled IL-2 offer a possible explanation for the lack of IL-2 responsiveness by demonstrating that no high affinity binding sites were present on activated T-ALL/NHL cells in three cases with high percentage of Tac-positive cells. Thus, the IL-2 receptor on IL-2 receptor positive malignant T cells is a low affinity receptor (defined by the anti-Tac antibody) which is not able to mediate IL-2 dependent growth. The data may indicate that the 75-kd chain of the receptor is missing on T-ALL/NHL cells since the expression of the Tac peptide alone is considered to be insufficient for growth induction in T cells (36–38).

To further document the absence of the 75-kd peptide, cross-linking studies of the IL-2 receptor using the anti-Tac antibody would be useful. These studies, however, would require cell numbers (10^{8} cells) which are obviously not available from patient samples.

Recent data by Rubin et al. (39) suggest that under certain conditions the expression of the Tac peptide alone is sufficient at least to transmit stimulatory signals by IL-2. Using mouse L cells transfected with the gene encoding the Tac peptide, ^{3}H-thymidine uptake and DNA synthesis could be demonstrated in response to IL-2 in individual cells. However, the percentage of responding cells has not been estimated, and IL-2 dependent growth has not been demonstrated.

In our experiments we were not able to confirm the results of Touw et al. (17), which have been obtained in a semisolid culture system, showing proliferation of malignant T cells lower (4–30-fold) than but comparable to normal T cells in response to mitogens and IL-2. They even found one case with a mature phenotype responding to PHA plus IL-2 in the same way as normal T cells. However, in repeated experiments we have not seen significant proliferation in our two patients with stage III ALL/NHL in response to IL-2.

In summary, our data argue against a role of IL-2 in T-ALL/NHL growth similar to the one for normal T cells. First, freshly isolated malignant T cells do not express IL-2 receptors, and IL-2 does not induce or maintian growth in vitro. Second, T-ALL/NHL cells induced by compounds thought to mimic physiological stimuli (PHA and phorbol ester) do express IL-2 receptors but do not show an increase in cell numbers in response to IL-2 as normal T cells do under the same conditions. Third, the IL-2 receptors on the malignant T cells investigated do not show high affinity binding characteristics and thus do not represent functionally active IL-2 receptors. It cannot be excluded, however, that intracellular IL-2 which may be produced by malignant T cells is active. In addition, certain culture conditions or conditions found in vivo may facilitate the additional expression of the 75-kd receptor protein. Thus, further studies are necessary to study the regulation of expression of the 75-kd protein to define the role of IL-2 in growth regulation of malignant T cells not induced by HTLV-I. Alternatively, other growth factors acting on T cells such as interleukin-4 have to be considered to be involved in the induction and maintenance of growth of malignant T cells (36, 37).

Acknowledgments. We thank Biogen S. A., Geneva, for providing r-IL-2, and Heidi Sauter for excellent secretarial assistance. This work was supported in part by a grant from the tumor centre Heidelberg/Mannheim to K-M. Debatin and P. H. Krammer. J. Fischer is a recipient of a scholarship from the German Cancer Research Centre.

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