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MODULATION OF LYMPHOKINE-ACTIVATED KILLER CELL–MEDIATED CYTOTOXICITY BY ESTRADIOL AND TAMOXIFEN

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The effect of tamoxifen (TX) and estradiol (E2) on interleukin-2 (IL-2)-activated killer (LAK) cell–mediated cytotoxicity was examined using spleen cells of Fisher 344 rats as the source of effectors and P815 murine mastocytoma cells as targets. Treatment of target cells with either TX or E2 for 4 or 18 hr rendered them highly sensitive to LAK cell–mediated lysis. When TX and E2 were applied jointly, cytotoxicity remained at the level of TX treatment alone. The cytotoxic potential of IL-2-primed LAK cells was not modified consistently by TX and E2. When TX-treated target and effector cells were combined, high cytotoxicity characteristic of sensitized target cells was observed. In similar experiments with E2-treated cells, both enhancement and inhibition of cytotoxicity by treated effector cells was seen in some designs. Target cells could be sensitized for LAK cell–mediated destruction by physiological concentrations (1 nM) of E2 and equimolar concentrations of TX. Sensitization led to the accelerated release of the nuclear label ^{3}H-thymidine from target cells after the cytotoxic insult and could be prevented by treatment with the metabolic inhibitors cycloheximide and actinomycin D. Enhanced ^{3}H-thymidine release from TX-treated targets was also demonstrated after induction of Ca^{2+} influx by exposure to the ionophore A23187. Neither E2 nor TX exerted a direct cytotoxic effect on P815 cells. P815 cells had no classical receptors for E2 or progesterone.

Treatment of normal lymphoid cells with interleukin-2 (IL-2) induces cytotoxic cells that are capable of killing various tumor targets. These IL-2-induced effector cells are called lymphokine-activated killer (LAK) cells. LAK cells have been tested in recent years for the treatment of various experimental and human tumors with encouraging results (Rosenberg *et al.,* 1987).

The non-steroidal estrogen antagonist tamoxifen (TX) is currently used for the treatment of estrogen-dependent breast carcinomas and of some other hormone-dependent tumors. The present consensus is that this drug interferes with the growth-promoting action of estradiol on breast cancer cells (Lerner and Jordan, 1990). However, it was observed that this drug has a beneficial effect on patients with estrogen receptor–negative tumors (Baral *et al.,* 1987). The reason for this is unknown at the present time. One possibility is that TX favorably influences the immune defence of patients against cancer. Indeed, earlier experiments from this laboratory and from others revealed that TX is a potent immunomodulatory agent (Baral *et al.,* 1994*a;* Nagy and Berczi, 1986) capable of amplifying killer cell–mediated destruction of tumor cells (Baral and Vanky, 1987; Baral *et al.,* 1994*b,* 1995). Here we present evidence that TX and estradiol are powerful enhancing agents of LAK cell–mediated cytotoxicity.

MATERIAL AND METHODS

Animals

Female Fischer (F344) rats weighing 150–170 g were obtained from Canadian Breeding Farm Laboratories (Montreal, Canada). All animals were maintained on a standard diet (Wayne's Laboratory Blocks, with 6% fat content, Chicago, IL) and on water supplied *ad libitum.* Animals were housed in a temperature- and humidity-controlled room with 12 hr light/dark cycles.

Cell lines

The natural killer (NK)-resistant mastocytoma P815 (Dunn and Potter, 1957) was used as an indicator of LAK activity. Cells were grown in RPMI-1640, supplemented with 5 μg/ml of insulin, 500 μM of sodium pyruvate, 50 IU/ml of penicillin, 50 μg/ml of streptomycin and 10% FBS (GIBCO, Grand Island, NY). For culturing LAK cells this complete medium was supplemented with 5×10^{-5} M 2-mercaptoethanol (Fisher, Orangeburg, NY) and is referred to as LAK medium.

Reagents

17-β-estradiol (E2) was obtained from Sigma (E-8875, Mississauga, Canada) and TX (code Fc-1157a) was a gift from Imperial Chemical Industries (Macclesfield, UK). Both drugs were initially dissolved in absolute ethanol (10^{-2} M) and appropriately diluted in tissue culture medium. The final concentration of ethanol was less than 0.1% in the cultures which had no effect on lymphocyte reactions. Human rIL-2 was obtained from ICN (Costa Mesa, CA). The specific activity of IL-2 was approximately 3×10^6 Biological Response Modifiers Program (BRMP) units per milligram of protein. One BRMP unit of IL-2 is equal to 2.25 international units. Activity was determined by half-maximal proliferation of IL-2-dependent CTLL-2 cells by the manufacturer.

Preparation of spleen cells

Spleens were removed aseptically and gently teased and the cells suspended in RPMI-10% FCS. Large debris was allowed to settle out at room temperature for 5 min. Lymphocytes were obtained after centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradients at 500 *g* for 20 min. Cells were washed twice in RPMI-10% FCS. Phagocytic cells were removed by incubation with iron powder (40 mg iron powder + 10 ml of cell suspension, 2×10^7 cells/ml) for 60 min at 37°C in a 5% CO_2 atmosphere followed by separation with a magnet. Non-adherent cells were washed and counted prior to use.

Generation of LAK cells

Spleen cells (2×10^6/ml) of F344 rats were cultured in medium supplemented with 500 IU/ml rIL-2 for 6–7 days at 37°C and 5% CO_2. Cells were washed twice and used as effector cells in cytotoxicity assays.

Cytotoxicity assay

Specific ^{51}Cr (Amersham, Oakville, Canada; specific activity: 250–500 mCi/mg Cr; 9.25–18.5 GBg/mg chromium) release from P815 cells was determined after 5 hr of incubation (37°C, 5% CO_2). Target and effector cells were pre-treated with TX (1 μM to 1 nM) and/or E2 (1 μM to 1 nM) for various times, then washed. Effector cells were then labeled with ^{51}Cr (100

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μCi/10^7 cells) for 1 hr and washed again, and 5×10^3 cells/100 μl were plated into each well of flat-bottomed microtiter plates (Nunc, Kumstrup, Denmark). Effector cells were added in 100 μl at indicated ratios and incubated in triplicate for 5 hr at 37°C in air containing 5% CO_2. After incubation, 100 μl of supernatant were harvested from each well and counted with a gamma counter (Beckman, Gamma 8000, Fullerton, CA). The percentage of specific release was calculated according to the formula:

$$\text{Percent cytotoxicity} = \frac{ER - SR}{TR - SR} \times 100 \qquad (1)$$

where ER is the experimental release. Spontaneous release (SR) was obtained from wells receiving target cells and medium only, and total release (TR) was obtained from wells receiving 1% Triton X-100. SR was always less than 10% of TR. Mean cpm ± SE of triplicate cultures was calculated and used for the evaluation of experiments. The *t* test was used for statistical analysis of the results.

^{3}H-thymidine release

DNA fragmentation was assayed by the release of ^{3}H-thymidine from P815 target cells according to the method of Kolber (1992). In some experiments, ^{3}H-labeled target cells were treated with the Ca^{2+} ionophore A23187 at 0.5 μM concentration for 2–4 hr, which was followed by quantitation of isotope release.

Metabolic inhibition

Cycloheximide (CH; Sigma), which inhibits protein synthesis, and actinomycin D (AD; Sigma), a DNA synthesis inhibitor, were used to treat target cells (CH: 10 μM, AD: 1 μM) for 30 min at 37°C in 5% CO_2, then washed 3 times with RPMI + 10% FCS. Cells were labeled with ^{51}Cr as described above and washed 3 times again with RPMI + 10% FCS. Labeled cells were then used in a cytotoxicity experiment at 1:25 target/effector ratio for 4 hr in the absence or presence of 1 μM TX or E2.

Receptor assays

The dextran-coated charcoal assay was used for both estrogen and progesterone receptors (EORTC Breast Co-Operative Group, 1980; Pichon and Milgrom, 1977). Both assays were performed in a buffer containing 0.01 M Tris-HCl, 0.0015 M EDTA, 10% glycerol and 2.5 mM dithiothreitol.

RESULTS

Pre-treatment (1 hr) with TX of target, effector cells or both cell types was not sufficient to obtain consistent results. One hour E2 treatment had little effect on the target and inhibited significantly effector cells (data not presented). Effector cells pre-treated with IL-2 for only 24 hr were able to exert significant cytotoxicity if both the targets and the effectors were also pre-treated with TX, E2 or both for 4 hr (Table I, Exp. 1). If pre-treatment with IL-2 of the effectors lasted 48 hr, it was sufficient to pre-treat only the target cells with TX or E2 to achieve a significant cytotoxic action. A similar degree of cytotoxicity was observed if TX-treated targets were combined with TX-treated effectors. Some enhancement of cytotoxicity was also seen when only the effector cells were pre-treated with TX. Effector cells treated with IL-2 for 6 days lysed drug-treated targets very efficiently, yet their cytotoxic action was inhibited by additional treatment with either TX or E2. Nevertheless, if drug-treated targets and effectors were combined, significant enhancement of cytotoxic action was maintained (Exp. 3). Similar results were obtained when drug treatment was extended to 18 hr.

The next series of experiments were performed with physiological concentrations (1 and 10 nM) of E2 and equimolar concentrations of TX. As shown in Table II, even at this concentration, E2 pre-treatment of target cells is capable of augmenting significantly LAK-mediated destruction. A higher concentration (100 nM) was more efficient in this respect. Both TX and E2 inhibited effector cell function at 100 nM concentrations but not at 10 or 1 nM. When both effector and target cells were treated with 1, 10 or 100 nM of TX, E2 or TX + E2, cytotoxicity was enhanced at levels similar to target treatment alone.

Experiment 1 of Table II is illustrated in Figure 1. It is clear that TX and E2 were most effective in enhancing the cytotoxic reaction at a 1:25 target:effector ratio. This was true when the

TABLE I – INFLUENCE OF ESTRADIOL AND TAMOXIFEN ON LYMPHOKINE-ACTIVATED KILLER CELL-MEDIATED CYTOTOXICITY

Experiment[1]	Treatment		Percent specific ^{51}Cr-release		
	IL-2	Drug[2]	Target treated	Effector treated	Both treated
1 (2)	24 hr	None	0	0	0
		TX 4 hr	3 ± 3	2 ± 2	23 ± 8.5[c]
		E2 4 hr	0	0	12 ± 4[c]
		TX + E2 4 hr	2 ± 2	3 ± 3	27 ± 7[c]
2 (2)	48 hr	None	3 ± 3	3 ± 3	3 ± 3
		TX 4 hr	65 ± 3[c]	13 ± 1[b]	51 ± 5[c]
		E2 4 hr	52 ± 16[c]	3 ± 0.5	14 ± 0
		TX + E2 4 hr	62 ± 13[c]	18 ± 6[b]	49 ± 9[c]
3 (2)	6 days	None	31 ± 11	31 ± 11	31 ± 11
		TX 4 hr	96 ± 4[c]	8 ± 3[a]	58 ± 10
		E2 4 hr	92 ± 7[b]	5 ± 0[b]	55 ± 9
		TX + E2 4 hr	95 ± 5[b]	14 ± 0[a]	67 ± 17

In each experiment 5×10^3 target cells were used in triplicate cultures. Target effector ratio was 1:25.–[1]Number of experiments performed for each version given in brackets. Mean % cytotoxicity ± S.E. calculated from all data available for a particular experimental design from replicate experiments.–[2]TX and E2 were applied at 1 μM concentrations in all experiments. *Statistics:* the *t* test was used for evaluation: [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. Superscripts a, b or c were used for the comparison of experimental values to controls. Spontaneous (S) and total (T) ^{51}Cr-release (cpm ± SE): Exp. 1/1, SR: 562 ± 25, TR: 5,793 ± 179; 1/2, SR: 438 ± 21, TR: 4,408 ± 7; 2/1, SR: 532 ± 5, TR: 5,491 ± 9; 2/2, SR: 468 ± 5, TR: 4,552 ± 29; 3/1, SR: 454 ± 7, TR: 4,614 ± 35; 3/2, SR: 390 ± 10. Spontaneous and total release from untreated (U) P815 target cells and from those treated with TX or E2 for 4 hr: *Exp. 1,* SR/U: 114 ± 5, SR/TX: 112 ± 4, SR/E2: 131 ± 1, TR/U: 1,882 ± 27, TR/TX: 1,916 ± 48, TR/E2: 1,896 ± 42; *Exp. 2,* SR/U: 220 ± 8, SR/TX: 231 ± 9, SR/E2: 230 ± 18, TR/U: 2,339 ± 28, TR/TX: 2,365 ± 30, TR/E2: 2,350 ± 20.

TABLE II – EFFECT OF NANOMOLAR CONCENTRATIONS OF E2 AND EQUIMOLAR CONCENTRATIONS OF TX ON LAK CYTOTOXICITY

Experiment[1]	Treatment		Percent specific ^{51}Cr-release		
	IL-2	Drug	Target treated	Effector treated	Both treated
1 (2)	6 days	None	17 ± 3	17 ± 3	17 ± 3
		100 nM TX	62 ± 2[c]	3 ± 1[a]	58 ± 0[c]
		100 nM E2	58 ± 2[c]	3 ± 1[a]	59 ± 1[c]
		100 nM TX + E2	69 ± 1[c]	4 ± 0[a]	62 ± 2[c]
2 (2)	6 days	None	16 ± 2	16 ± 2	16 ± 2
		10 nM TX	55 ± 7[b]	20 ± 0	13 ± 3
		10 nM E2	21 ± 3	15 ± 5	37 ± 1[c]
		10 nM TX + E2	60 ± 2[c]	13 ± 3	59 ± 3[c,*]
3 (2)	6 days	None	6 ± 0.5	6 ± 0.5	6 ± 0.5
		1 nM TX	10 ± 0	13 ± 3	14 ± 4
		1 nM E2	30 ± 4[b]	18 ± 2[b]	22 ± 2[c]
		1 nM TX + E2	22 ± 2[b]	12 ± 2	11 ± 1
4 (2)	7 days	None	13 ± 1	13 ± 1	13 ± 1
		1 nM TX	23 ± 5	13 ± 3	24 ± 10
		1 nM E2	61 ± 7[c]	12 ± 2	34 ± 6[a]
		1 nM TX + E2	19 ± 5	12 ± 2	12 ± 2

Please see legend to Table I.–[1]Number of experiments performed for each design is given in brackets. Incubation with drugs was done for 4 hr prior to cytotoxicity testing in all experiments. *Statistics:* please see legend to Table I. *Comparison of TX treatment with TX + E2: $p < 0.001$. Spontaneous (SR) and total (TR) ^{51}Cr-release (cpm ± SE): Exp. 1/1, SR: 328 ± 9, TR: 3,493 ± 26; 1/2, SR: 413 ± 5, TR: 4,143 ± 31; 2/1, SR: 324 ± 6, TR: 3,172 ± 64; 2/2, SR: 323 ± 5, TR: 3,409 ± 17; 3/1, SR: 325 ± 2, TR: 3,190 ± 41; 3/2, SR: 357 ± 5, TR: 3,503 ± 4; 4/1, SR: 313 ± 5, TR: 3,085 ± 76; 4/2, SR: 354 ± 8, TR: 3,528 ± 31.

TABLE III – PRE-TREATMENT OF P815 CELLS WITH CYCLOHEXIMIDE OR ACTINOMYCIN-D INTERFERES WITH SENSITIZATION FOR CYTOLYSIS

Treatment	Percent specific lysis		
	No pre-treatment	Cycloheximide	Actinomycin-D
None	16 ± 1	2 ± 0.5	2 ± 1.0
TX	42 ± 6	2 ± 1.0	3 ± 1.0
E2	38 ± 3	1 ± 0.5	3 ± 0.5

Target/effector ratio = 1/25.

TABLE IV – TAMOXIFEN POTENTIATES LAK CELL–INDUCED RELEASE OF ^{3}H-THYMIDINE FROM P815 TARGET CELLS

Treatment[1]	Percent ^{3}H-thymidine release			
	Exp. 1	Exp. 2	Exp. 3	Mean ± SE
Control	26	21	22	23 ± 1.5
Tamoxifen	49	48	43	47 ± 1.8[2]

[1]Target: effector cell ratio = 1:25. P815 cells were labeled with ^{3}H-thymidine (100 μCi/ml for 18 hr), as described by Kolber (1992), and pre-treated with tamoxifen (1 μM) for 4 hr, as in previous experiments. The cytotoxic reaction was terminated after 4 hr of incubation. Spontaneous isotope release ranged from 279 to 319 cpm and total release from 2,280 to 3,114 in the various experiments.–[2]Release of ^{3}H-thymidine from TX-treated cells, compared to controls by *t* test: $p < 0.01$.

target was treated alone or when both target and effector cells were treated. Similar observations were made in all other experiments.

Pre-treatment of P815 cells with actinomycin-D, which inhibits nucleic acid synthesis, or with cycloheximide, which inhibits protein synthesis, interfered with TX- and E2-induced sensitization and inhibited the lysis of otherwise untreated P815 cells by LAK effectors. These results indicate that P815 cells actively participate in the process of sensitization (Table III). Additional experiments revealed that the release of the nuclear label ^{3}H-thymidine was also enhanced by TX (Table IV), which suggests DNA fragmentation. When TX-treated targets were exposed to the calcium ionophore A23187 at 0.5 μM concentration for 2–4 hr, an enhanced release of ^{3}H-thymidine, indicating accelerated nuclear breakdown, was observed (Table V).

TABLE V – INCREASED RELEASE OF ^{3}H-THYMIDINE FROM TX-TREATED P815 CELLS AFTER EXPOSURE TO THE Ca^{2+} IONOPHORE A23187

Experiment[1]	TX treatment (hr)	A23187		Percent ^{3}H-thymidine release
		μM	hr	
1 (2)	—	0.5	2	9 ± 0
	4	0.5	2	15 ± 3[2]
2 (2)	—	0.5	4	13.5 ± 0.5
	4	0.5	4	19.5 ± 0.5[2]
3 (2)	—	0.5	2	0 ± 0
	16	0.5	2	5.5 ± 0.5[3]
4 (2)	—	0.5	4	0 ± 0
	16	0.5	4	15 ± 1[4]

Tamoxifen (TX) was used at 1 μM concentration in all experiments. [1]Results represent means ± SE for 2 separate experiments. *Statistics:* [2]$p < 0.05$, [3]$p < 0.01$, [4]$p < 0.001$.

To rule out further the cytotoxic effect of these drugs on the target cells, we examined the effect of TX and E2 on thymidine incorporation by P815 cells. The results, presented in Table VI, indicate that these agents, whether used separately or combined, exerted a slight inhibition of thymidine incorporation on day 1 and E2 also on day 3. Nevertheless, these experiments indicate that the target cells are capable of proliferating in the presence of these agents.

In the charcoal binding assay, P815 preparations bound 0.7 fM E2/mg protein (positive: over 3 fM/mg) and 9.3 fM of progesterone/mg protein (positive: over 15 fM/mg). Both values are in the negative range.

DISCUSSION

Our results indicate that the non-steroidal estrogen antagonist agent TX and estradiol itself both have the capacity of sensitizing the P815 target cells for LAK cell destruction. This sensitizing effect is highly significant and can be observed even at physiological concentrations of estradiol. In women, plasma E2 concentrations vary between 60 and 7,000 ng/l (0.22–25.7 nM), being the lowest during the early follicular phase, reaching peak values during the late follicular phase and subsiding to intermediate levels (7.3 nM) in the mid-luteal period (Ross *et al.*, 1981).

TABLE VI – EFFECT OF TX AND E2 ON ^{3}H-THYMIDINE INCORPORATION BY P815 CELLS

Treatment	^{3}H-thymidine incorporation		
	Day 1	Day 2	Day 3
Control	772,457 ± 1,238	194,801 ± 9,487	306,647 ± 9,503
TX	352,480 ± 1,235[2]	182,686 ± 2,112	284,826 ± 2,170
E2	262,648 ± 8,479[2]	190,113 ± 1,613	244,575 ± 1,163[1]
TX + E2	364,040 ± 6,583[2]	211,795 ± 2,448	282,548 ± 3,764

TX and E2 were applied at 1 μM concentration. *Statistics:* Drug-containing cultures compared to control: $^1p < 0.05$, $^2p < 0.001$.

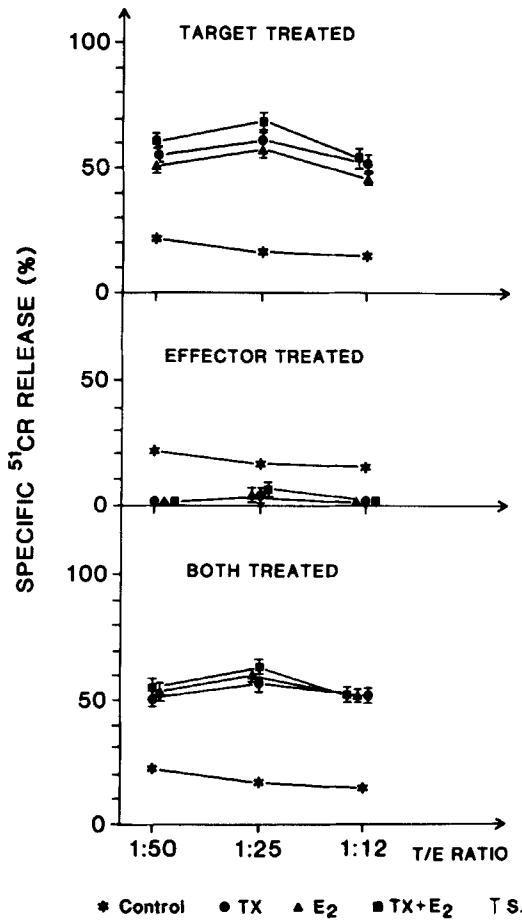

FIGURE 1 – Lymphokine-activated killer activity of rat spleen cells treated with human recombinant IL-2 for 6 days. P815 cells were used as targets. TX and E2 were used at 100 nM concentrations for pre-treatment for 4 hr.

In all experiments with untreated target cells, lysis was in direct correlation with the number of effector cells used. However, altered kinetics of lysis were observed with drug-treated targets in that maximal isotope release occurred at 1:25 target:effector ratios rather than at 1:50, with a decrease to 1:12 in most, but not all, experiments. This altered kinetics was present with TX, E2 or joint drug treatment and when both target and effector cells were treated (Fig. 1). LAK cells are capable of killing their targets by perforin-mediated membrane damage or by triggering programmed cell death (apoptosis). Apoptotic signaling of target cells is mediated by the Fas membrane receptor, which is triggered by a tumor necrosis factor (TNF)-like membrane-bound molecule on the surface of killer cells. Only the latter pathway is inhibited if transcription or translation is blocked in the target cell. DNA fragmentation is characteristic of apoptotic cell death (Zychlinsky *et al.*, 1991; Doherty, 1993). Cells such as cortical thymocytes, which are primed for apoptosis, are readily killed by Ca^{2+} ionophores (Arends and Wyllie, 1991). Therefore, it is likely that E2 and TX program P815 cells for apoptosis, which facilitates the delivery of the lethal hit by LAK cells. The fact that only one of the 2 pathways by which LAK cells can kill their targets has been enhanced by E2 and TX explains the altered, non-linear cytotoxicity curve that was obtained. In all probability, a high density of killer cells can destroy the targets by overwhelming membrane attack, leaving less or no time for active participation in the lytic process. However, during a weak cytotoxic reaction, there is apparently a more extensive active participation by target cells, which can be amplified by drug treatment. As a matter of fact, such altered curves can be generated without target cell treatment, simply by varying the effector: target ratio using filler spleen cells (Sabbadini, 1970). Similar enhancement of cytotoxicity was observed with TX- and E2-treated target cells using NK cells and cytotoxic T lymphocytes as effectors (Baral *et al.*, 1994*b*, 1995).

In rats, TX may have estrogenic or anti-estrogenic effects, whereas in mice it is estrogenic (Jordan *et al.*, 1980). Thymus-derived lymphocytes bearing the CD8 marker, which include killer cells, do express estrogen receptors (Cohen *et al.*, 1983; Stimson, 1988). However, we were unable to find classical estrogen receptors in the murine P815 cell line. This indicates that the programming of these cells for lysis was mediated by another, as yet unidentified, receptor for E2. The facts that physiological concentrations of E2 were effective in this respect and that active cell metabolism was required for target cell sensitization indicate that the sensitizing effect of E2 is probably mediated by a novel receptor.

That TX is capable of enhancing cell-mediated cytotoxicity *in vitro* was first described by Mandeville *et al.* (1984), who performed NK-mediated cytotoxicity assays with human peripheral blood lymphocytes using the K-562 erythroleukemic cell line as target. When TX was present during the assay, significant enhancement of cytotoxicity was observed by TX from 8 different donors, 10^{-7} and 10^{-8} M concentrations being effective. TX had no cytotoxic effect on the K-562 target cells. An additive effect in the augmentation of cytotoxicity was observed when the cells were exposed simultaneously to human leukocyte interferon and TX. These results were interpreted as showing that TX can directly stimulate the NK activity of normal human peripheral lymphocytes.

Our earlier observations (Baral and Vanky, 1987) indicate that the augmentation of cell-mediated cytotoxicity by TX may bear relevance to the immunobiology of tumors. Treatment with TX of peripheral T lymphocytes from patients with various types of carcinoma and with malignant mesenchymal tumors induced and/or augmented the destruction of autologous tumor and K562 target cells in a significant proportion of cases.

It was concluded in a recent review of the effect of TX on the immune response (Baral *et al.*, 1994*b*) that TX has an anti-proliferative effect on lymphocytes and inhibits the induction of humoral and cell-mediated immune reactions. TX is also inhibitory for suppressor T lymphocytes, and in some situations this could lead to enhanced immunoglobulin secretion by

B cells. TX antagonizes the stimulatory effect of E2 on phagocytosis, inhibits giant cell formation by monocytes and blocks H_2O_2 production by human neutrophils. In contrast, the LPS-induced production of TNFα by normal human monocytes and by rat peritoneal cells was enhanced by TX.

Killer and suppressor T cells ($CD8^+$) and NK cells express classical estrogen receptors; therefore, TX may act on these receptors as an anti-estrogen as exemplified in our experiments. However, the anti-proliferative effect of TX on lymphocytes is not dependent on the classical estrogen receptor. Current evidence indicates that the sensitizing effect of TX on ER^- target cells for cell-mediated lysis is mediated by another receptor on which E2 has a similar effect to that of TX. This receptor is present in Con A–activated lymphocytes, in the Nb2 rat lymphoma (Baral *et al.,* 1994*b*), in the Yac-1 and SL2-5 murine lymphomas, in the P815 murine mastocytoma, in the K562 human erythroleukemia cell line and in the majority of human ovarian carcinoma cells (Baral *et al.,* 1994*a,b*).

TX decreased serum prolactin (PRL), growth hormone (GH) and insulin-like growth factor-I levels, influenced the expression of hormone receptors and their binding proteins, which also affects the immune system. TX induced immunosuppression in rats, which could be reversed by additional treatment with either GH or PRL, suggesting that these hormones are effective antagonists of TX-induced immunosuppression. TX also antagonizes the stimulatory effect of PRL on lymphoid cells *in vitro* (Baral *et al.,* 1996).

In conclusion, our present results and earlier observations by us and by others indicate that estradiol and the anti-estrogenic agent TX have a significant potentiating effect on LAK cell activity, which is due to target cell sensitization for apoptotic lysis.

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