Eur J Cancer Clin Oncol, Vol. 20, No. 7, pp. 983–985, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Letter to the Editor

In Vitro Stimulation of Human NK Activity by an Estrogen Antagonist (Tamoxifen)*

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NATURAL killer (NK) cells comprise a subpopulation of large granular lymphocytes of distinct morphology, surface characteristics and biological activity (for review see ref. [1]). Recently, in our laboratory we have demonstrated that NK activity is an individual trait that varies little under physiological conditions [2] and can be modulated by a variety of agents [3, 4], including bacterial (*Bacillus Calmette-Guerin, Corynebacterium parvum* and *Brucella abortus*) and viral (influenza and poliovaccines) adjuvants, as well as interferon (IFN) and interleukin-2 (IL-2). As part of our ongoing studies on the mechanism of action and metabolic requirements for NK modulation, we have demonstrated that boosting of NK activity requires (a) the *de novo* synthesis of RNA and cellular proteins and (b) the integrity of the cell surface membrane. To date, the major internal mediators of positive regulation appear to be IFN and IL-2 [1, 5–7]. These mediators may be involved in different ways when NK activity is influenced by bacterial or viral adjuvants. It has also been suggested that IFN and IL-2 have a synergistic boosting effect on NK, with IFN inducing IL-2 receptors on mouse spleen cells [8]. This preliminary study describes the augmentation of NK activity by an antiestrogen, tamoxifen. In our experiments Ficoll-Hypaque-purified lymphocytes could repeatedly be stimulated with tamoxifen regardless of the sex and age of the donor. This observation might be of potential clinical importance since TAM is now used routinely in the treatment of estrogen-receptor-positive breast cancer patients [9–11] as well as endometrial [12], prostatic and renal cell carcinomas [13].

Tamoxifen (Nolvadex, ICI Ltd) is a non-steroid antiestrogen belonging to the triphenylethylene class of compounds. The purified powder was kindly provided by Dr W. Baumgarten (Stuart Pharmaceuticals, Wilmington, DE). It was solubilized in ethanol and diluted to 10^{-5} M/ml in RPMI-1640, then stored at −20°C until further use. Peripheral blood lymphocytes (PBL) were obtained from 8 normal donors aged 20–40 yr. All were in apparent good health and took no medication during the study period. PBL were treated with carbonyl iron to remove phagocytic cells, then layered on a Ficoll-Hypaque gradient. NK activity was evaluated by a standard overnight ^{51}Cr-release assay using K-562 erythroleukemic cell lines as targets [2]. Treatment of lymphocytes with either TAM, IFN or IL-2 was carried out by adding each agent directly to the test system. For TAM we used five different concentrations (10^{-6}–10^{-10} M/ml) of this drug. Human leucocyte interferon (IFN) was a gift from Dr J. L. Virelizier (Hôpital des Enfants Malades, Paris) and was used at 5 U/ml concentration. IL-2, also known as human T-cell growth factor, was purchased (Cellular Products Inc., Buffalo, NY) as a crude preparation and 10^{-2} dilution was used in this study. Statistical analysis of data was performed using ANOVA (analysis of variance) and Tuckey's test for comparison between specific pairs of means [14].

Data presented in Table 1 clearly show that *in vitro* treatment of purified PBL with TAM resulted in a significant augmentation of the NK cytotoxicity in the eight different donors studied. This augmentation was dose-dependent since

Accepted 21 December 1983.
*This study was supported by the Cancer Research Society Inc., and by ICI Pharma, Canada.
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10^{-6}, 10^{-9} and 10^{-10} mol/ml of TAM had no effect, while 10^{-7} and 10^{-8} mol/ml had a maximal effect. A dose–response curve could be elicited in all of the donots studied regardless of sex or age.

It was also noted that TAM had no direct effect on target cells since K-562 cells were not killed by TAM (10^{-6} mol/ml) in the overnight chromium release assay.

Moreover, as shown in Table 2, PBL from individual donors treated with either IFN (5 U/ml), IL-2 (10^{-2} dilution) or TAM (10^{-8} mol/ml) demonstrate a comparatively similar level of stimulation. These results were significantly different ($P < 0.05$) when compared to the normal unstimulated level of NK activity in the same individual (Tuckey's test). Using the same *ad hoc* test, we could demonstrate an additive effect of treatment when lymphocytes were simultaneously treated with TAM and IFN (Table 2), suggesting that there is no competition between these two agents for the cell-surface receptor.

TAM is a potent non-steroidal antiestrogen compound (estrogen antagonist) and acts by competing with estradiol for the high-affinity cytoplasmic estrogen receptor in tumor cells and subsequently binds to it [9, 10]. The newly formed complex translocates into the nucleus in the form of a receptor–antiestrogen and tends to remain there much longer than receptor–estradiol complexes. It thus suppresses the replenishment of cytosol receptors and renders the cell less responsive to subsequent estradiol effect [11]. However, is the action of TAM restricted to the so far known target cells? In this respect the results we are presenting clearly indicate that TAM can directly stimulate the NK activity of normal human peripheral blood lymphocytes.

TAM has been reported to affect the cellular metabolism of certain tumor cells in long-term tissue culture [15] by inhibiting the incorporation of [^{3}H]-thymidine. It seems unlikely, however, that TAM-augmented NK activity is due to a direct cytotoxic effect on target cells since K-562 cells were not killed by TAM (10^{-6} mol/ml) in the 16-hr assay. We are conducting an extensive investigation to identify the precise mechanism(s) of action of TAM. Whether TAM mimics the action of interferon on NK cells or acts directly at

Table 1. Boosting of human NK cell activity by tamoxifen

Lymphocyte source	Effect of tamoxifen (mol/ml) present throughout the culture period					
	None	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Donor 1	14 ± 1.2*	14 ± 1.6	17 ± 0.4	21 ± 2.1	15 ± 3.4	13 ± 1.2
Donor 2	9 ± 2.3	15 ± 2.6	15 ± 1.5	17 ± 1.9	14 ± 3.1	14 ± 0.3
Donor 3	16 ± 3.2	20 ± 0.9	23 ± 0.8	26 ± 1.2	24 ± 1.1	20 ± 0.8
Donor 4	20 ± 1.8	27 ± 1.7	29 ± 1.5	30 ± 0.7	24 ± 2.0	22 ± 1.0
Donor 5	18 ± 1.8	28 ± 3.0	33 ± 0.7	33 ± 0.2	35 ± 6.1	28 ± 3.0
Donor 6	48 ± 0.7	52 ± 5.7	56 ± 4.8	56 ± 5.4	56 ± 2.1	52 ± 0.1
Donor 7	22 ± 0.6	24 ± 2.1	30 ± 0.8	32 ± 0.8	32 ± 0.4	30 ± 0.3
Donor 8	23 ± 0.3	29 ± 1.7	42 ± 0.1	42 ± 0.8	38 ± 1.7	35 ± 2.3

*NK activity was tested in an overnight chromium release assay using K-562 cells as targets and is expressed in % lysis (mean ± SEM, $n = 3$) at the E/T ratio of 10/1. Statistical analysis of data (Tuckey's test) showed that treatment with TAM (10^{-8} and 10^{-9} mol/ml in most cases) significantly increase NK activity ($P < 0.05$).

Table 2. Comparative effect of treatment with tamoxifen, interferon, interleukin-2 and combined treatment with tamoxifen and interferon

Lymphocyte source	*In vitro* treatment				
	None	IFN (5 U/ml)	IL-2 (10^{-2} dilution)	TAM (10^{-8} mol/ml)	TAM + IFN
Donor 1	14 ± 1.2*	20 ± 0.6	20 ± 0.3	21 ± 2.1	24 ± 1.3
Donor 2	9 ± 2.3	16 ± 1.8	15 ± 0.8	17 ± 1.9	28 ± 2.8
Donor 3	16 ± 3.2	22 ± 2.6	21 ± 0.9	26 ± 1.2	29 ± 1.1
Donor 4	20 ± 1.8	27 ± 1.2	29 ± 1.5	30 ± 0.7	32 ± 1.7
Donor 5	18 ± 1.8	32 ± 3.5	31 ± 5.2	33 ± 5.2	55 ± 2.0
Donor 6	48 ± 0.7	57 ± 4.1	55 ± 2.8	56 ± 5.4	60 ± 2.1
Donor 7	22 ± 0.6	30 ± 2.2	33 ± 1.2	32 ± 0.8	37 ± 1.1
Donor 8	23 ± 0.3	35 ± 4.3	39 ± 0.8	42 ± 1.2	48 ± 1.9

Statistical analysis of data from each donor showed a significant difference ($P < 0.001$) between the five types of treatments (ANOVA analysis of variance). Then, using Tuckey's test, we found that treatment with IFN, IL-2 and TAM are similar and at the same time significantly different from normal values, and that treatment with TAM + IFN is also significantly different from the other four treatments ($P < 0.05$).

the level of the plasma membrane remains to be established. However, these preliminary results, as well as recent reports by others [16, 17], tend to point to the fact that hormones are among the most important environmental factors that influence NK cell function.

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