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EFFECT OF TOREMIFENE ON THE ACTIVITY OF NK-CELLS IN NZB/NZW MICE

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Summary—The effect of toremifene on NK-cells isolated from the spleen of NZB/NZW mice was studied in comparison to tamoxifen and estradiol. Unlike estradiol but like tamoxifen, toremifene did not influence the activity of NK-cells. Low doses (0.1 and 10.0 mg/kg) of toremifene did not suppress, but a high dose of toremifene and tamoxifen (50 mg/kg for 6 weeks) suppressed the stimulating effect of human interferon alpha on the cells.

INTRODUCTION

NK-cells (Natural Killer cells) are specialized lymphocytes which account for about 3% of all the lymphocytes in the blood [1] and which seem to have a cytotoxic effect on various tumor cells as well as on some microorganisms and virus-infected cells without any prior sensitization [2, 3]. Thus, they are an important part of the host's defense mechanism, possibly as the first weapon against disease. However, the origin and the whole extent and biological significance of their functions are still partly unclear.

The activity of NK-cells is regulated by numerous positive and negative signals. In many studies, interferons have been shown to increase the activity of NK-cells [4, 5], a negative effect has been observed with e.g. prostaglandins [6, 7]. Evidently, the NK-cells themselves have an immunoregulatory effect as well [7]. Estradiol in continuous high concentrations has been observed to decrease the activity of NK-cells in NZB/NZW mice, which otherwise have a high NK-activity [8, 9]. On the basis of this observation, the question has been raised about the effect of antiestrogens, for instance toremifene, on NK-cells. Toremifene is a new synthetic triphenylethylene antiestrogen compound specially aimed at breast cancer treatment. It has both estrogen receptor-mediated and estrogen receptor-independent antitumor effects [10, 11].

The present study was carried out to investigate the effect of toremifene on the activity of NK-cells isolated from the spleen of NZB/NZW mice. The reference substances were tamoxifen and estradiol. In addition, the stimulating effect of human interferon alpha was studied. Mouse YAC-1 cancer cells which were labeled with radioactive chromium were used as target cells for the NK-cells. Thus the activity of the NK-cells can be measured directly on the basis of the radioactivity released from the destroyed target cells.

MATERIALS AND METHODS

Experimental animals and dosing

5–6-week old female NZB ♂/NZW ♀ F1 hybrid mice were purchased from Bomholtgaard, Copenhagen, Denmark and kept in quarantine for 1 week. They were then administered toremifene or tamoxifen (both from the Chemical Research Laboratory of Farmos Group Ltd, Medipolar, Oulu, Finland) 0.1, 1.0, 10.0 or 50.0 mg/kg per os or 17β-estradiol (Sigma, St Louis, Mo., U.S.A.) 1.0 mg/kg i.p. 5 times a week for 6 weeks. The control group was administered a water solution which contained polyethylene glycol (PEG 3000, 2.88%) and Tween 80 (0.19%). This solvent was also used as the vehicle for the antiestrogens. About 18 h before killing, some of the control animals were given a single dose of 9×10^5 IU of interferon alpha which was kindly donated by Professor Kari Cantell from the National Health Institute in Finland.

NK-cell cytotoxicity test

After 6 weeks administration of the test-compounds the mice were killed in a CO_2-chamber and the NK-cells were isolated from the spleens as described earlier [12]. Briefly, spleens were manually homogenized in ice-cold medium (RPMI 1640 medium, KC-Biological, Lenexa, Kans, U.S.A.; supplemented with 10% heat inactivated newborn calf serum, Gibco Europe Ltd, Renfrewshire, Scotland; 300 mg L-glutamine l^{-1}, Fluka AG, Buchs, F.R.G.; 10 mM HEPES buffer, Sigma, St Louis, Mo., U.S.A. and 10 μg gentamycin ml^{-1}, Sigma). Large granular lymphocytes (LGL-cells), including the NK-cells [1] were isolated from the spleen cell suspension by centrifugation on Percoll-paque gradients (Pharmacia Fine Chemicals, Uppsala, Sweden). Cells collected from the interface were washed and suspended in cold medium, and counted in a Bürkerchamber.

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YAC-1 cells, kindly provided by Professor Eero Saksela, University of Helsinki, Department of Pathology, were cultured in suspension in the same medium. The cells (5×10^6) were labeled with 100 μCi of $Na_2^{51}CrO_4$ (Amersham, Buckinghamshire, England) for 1.5 h in 1 ml of serum.

In the ^{51}Cr-release cytotoxicity assay the YAC-1 cells (6250 cells in 0.1 ml of medium) were pipetted to the 96-microwell plates (Nunc, Røskilde, Denmark) together with the isolated NK-cells (5×10^5 cells in 0.1 ml of medium), i.e. in an effector-target ratio of 80:1. To control the spontaneous lysis of the target cells, they were also incubated with medium only. The maximum release of ^{51}Cr was achieved after incubation of the target cells in 10% Triton-X-100. After incubation, 100 μl of supernatant from each well was collected and counted for 10 min in a gamma counter (1275-002 MiniGamma LKB Wallac, Turku, Finland).

RESULTS

Toremifene treatments did not significantly decrease the NK-cell activity in NZB/NZW mice (Fig. 1): cytotoxicity varied somewhat below or above control values, and did not show any dose dependence. On the contrary, there was a statistically significant difference between the effect of toremifene and that of estradiol on the NK-activity (Fig. 1). Similar results were obtained with tamoxifen: no significant difference could be observed between the effect of tamoxifen and that of toremifene.

The variation inside the groups is relatively wide, probably because the normal activity level of NK-cells varies between individuals. The mean percentage lysis (and SD) for the 30 control samples, calculated as percentage of maximal lysis of each experimental series, is 13.0 ($\pm$3.6).

As reported earlier [4, 5], the activity of NK-cells was increased above control by a single dose of interferon alpha. This also shows that the NK-cells had conserved their biological activity during the tests and that the results obtained were not caused by an unspecific effect of test conditions on the NK-cells.

The effect of toremifene on interferon-stimulated NK-cell activity was also studied after 6 weeks toremifene administration (doses 0.1, 10 and 50 mg/kg) followed by a single dose of interferon (9×10^5 IU). At low doses toremifene did not depress the stimulating effect of interferon alpha, the values being 209 $\pm$ 39, 196 $\pm$ 47 and 179 $\pm$ 69 percentages of the control after interferon only and toremifene 0.1 mg/kg and 10.0 mg/kg, respectively. A high dose of toremifene and tamoxifen (50 mg/kg for 6 weeks) suppressed the interferon stimulation so that NK-cell cytotoxicity remained at the control level.

DISCUSSION

Estrogens are known to depress most of the major functions of the cell-mediated immune system [9]. In NZB/NZW mice β-estradiol has been shown to depress NK-activity [8]. In humans NK-cell activity fluctuates along with the menstrual cycle and suggests

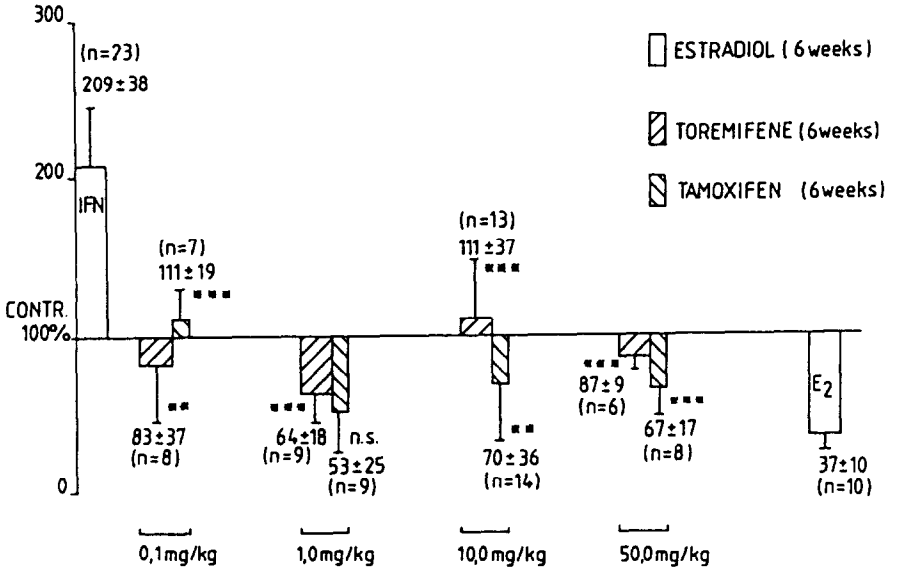

Fig. 1. The effects of interferon alpha (IFN), estradiol (E_2) and different doses of toremifene and tamoxifen on NK-cell activity in NZB/NZW mice. The results are given as percentage of the lysis ($\pm$SD) of the vehicle-treated control animals, the control being rated at 100%. The mean percentage lysis ($\pm$SD) per 30 control samples is 13.0 ($\pm$3.6) of the maximal release. The different doses of toremifene and tamoxifen are given under the columns. Estradiol was given at a dose of 1.0 mg/kg for 6 weeks and IFN as a single dose of 9×10^5 IU 18 h before killing. ** and *** indicate a statistically significant difference ($P < 0.01$ and $P < 0.001$, respectively, by Student's t-test) compared with the estradiol treated group. NS = not significant.

an inverse correlation of NK-activity with circulating estrogen levels [13]. The results of this study indicate that the activity of the NK-cells in NZB/NZW mice is not decreased by antiestrogen treatments, the effect of different doses of both toremifene and tamoxifen (ranging from 0.1 to 50 mg per kg) being much the same. Indeed, when these levels of NK-activity were compared to the activity after estradiol treatment, there was a statistically significant difference in all cases except one (see Fig. 1). This is an important observation with regard to cancer treatment as the NK-cells are quite evidently an essential part of body's own defence system against cancer. The only suppressive effect of toremifene on NK-cells was found after 6 weeks' treatment with a high dose (50 mg/kg), the NK-cell activity remaining at the control level in spite of interferon stimulation.

The data concerning the correlation between NK-cell activity and malignancy in humans are still contradictory. It has been stated that patients with neoplastic disease show a general reduction of NK-cell activity in comparison with healthy controls, the activity being further reduced in patients with advanced disease [14, 15]. Garner *et al.*[16] investigated the presurgical NK-activity in patients with benign and malignant breast disease receiving no therapy, and observed a close correlation between decreasing NK-cytotoxicity and increased spread of tumor. The patients with benign disease had the same NK-cytotoxicity values as healthy controls. In contrast to the above observations, Brenner and his coworkers [17] showed that NK-activity in breast cancer patients is elevated when compared to healthy women. However, after chemotherapy the NK-cell activity decreased clearly below the control values. Different therapeutic modalities were compared in terms of lymphocyte recovery and NK-cell activity. Only tamoxifen-treated patients demonstrated levels of NK-activity that were not statistically different from the levels observed in untreated cancer patients. Yet the data were obtained mostly from short-term follow-up studies.

In a recent study (R. Valavaara, A. Toivanen and E. Nordman, to be published) patients with advanced breast cancer and continuous toremifene treatment were followed up to 1 yr. No significant effect on the NK-cell activities were found. These data together with the results presented here support the beneficial effects of toremifene therapy in steroid hormone sensitive cancers.

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