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# Immunotherapy of Cancer—A Historical Note

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## Abstract

**We examined the possibility that the anti-estrogens, tamoxifen (TX) and toremifen (TO) interacted with the immune system. Indeed, both TX and TO stimulated cells mediated cytotoxicity reactions by various killer cells: killer T (TK), natural killer (NK), lymphokine activated killer (LAK) cells. Both TX and TO inhibited the growth of tumors that express estrogen receptors. Thus these anti-estrogens inhibited tumor growth and stimulated killer cells for cytotoxicty on such tumors. Therefore these agents were presumed to stimulate tumor immunity. We tested the P815 mouse mastcytoma with TK, LK, and TX or TO. A therapeutic effect was observed in both experiments. The SL2-5 murine lymphoma was tested with NK and TX cells or TO cells and successful immunotherapy was observed. We digested human breast carcinomas and lung tumors with collagenase. The killer cells were separated from tumor cells on Ficoll gradients. TX and TO enhanced the cytotoxic effect of autologous killer cells on the corresponding tumor cells. This experiment indicates that the results obtained in animals are also valid for human malignant disease.**

## Keywords

**Murine Tumors: P815 Masocytoma, SL2-5 Lymphoma, Human Cancers: Breast Carcinomas and Lung Carcinomas, Tamoxifen, Toremiphen, Thymus-Derived Lymphocytes, Killer T Cells (TK Cells), Natural Killer Cells (NK Cells), Lymhokine Activated Killer Cells (LAK Cells), Combination Immunotherapy of Cancer**

## 1. Introduction

Cancer immunity became common knowledge during the 1970s. The first book has been published on the subject by Harris and Sinkovics in 1970 [1]. By that time it was clear that tumors induced by carcinogenic agents or

by viruses had antigens. Individually specific antigens were characteristic of chemically induced tumors, whereas virus induced tumors expressed cross-reactive viral antigens but also had individually specific antigens.

Tumors also expressed embryonic antigens. A famous embryonic antigen was the carcinoembryonic antigen of the human digestive system, discovered by Krupey, Gold and Freedman [2]. With this knowledge the dialogue started whether or not anti-tumor immunity could be used for immunotherapy. Progress was slow. Only a few laboratories worked in the field. The investigations were challenging due to the complexity and fragility of tumors.

T cell immunity was acknowledged in the 1970s [3]. Delayed type hypersensitivity to chemically induced tumors was described by Oettgen *et al*. [4]. George Klein and colleagues analyzed tumor escape from immune control [5]. That suppressor T cells also contributed to tumor escape was discovered by my colleagues in the same laboratory in Winnipeg, Shigeyoshi Fujimoto [6].

Borsos and colleagues reported that chemotherapeutic agents enhanced the killing of tumor cells by antibodies and complement [7]. I remembered this paper when we designed our experiments. Theoretically this is an important paper.

Nowotny investigated the anti-tumor effects of lipopolysaccharides (LPS) [8]. Hershy and McLennon described the anti-tumor effects of macrophages [9].

The therapeutic effect of immune action was established by the intra lesional injection of living bovine bacillus (BCG) to intradermal tumors which produced cure of the cancer [10].

Real immunotherapy by immunization, which would induce effective cancer immunity was not established at this time.

## 2. Results

The story starts with the discovery that tumor infiltrating lymphcytes grow out in tissue culture and are able to kill autologous or syngeneic tumor cells. This phenomenon was seen first with the methycholantrene induced MCD tumor of strain 13 guineaa pigs. In tissue culture of MCD small round cells appeared. The round cells formed blust-like cells and quickly destroyed the monolayer cancer cells. If the killer cells were not stimulated by cancer cells they stopped growing and slowly disappeared. These cells needed the cancer cells for growth and they exerted cytotoxicity. It was theorized that tumor infiltrating T cells grew out in culture [11] [12]. Morphologically the killer cells looked like T cells and T cell antigens were detected [13]. So tumor infiltrating T cells can grow out from the tumor in tissue culture. This experiment could be repeated at will.

Immunotherapy experiments were done with killer cells grown in culture and the syngeneic MCD tumor [11] [12]. The results of these therapy experiments were disappointing. Only minor inhibition was achieved with these anti-tumor effector cells [13]. This was unexpected because of the excellent cytotoxic effect of these effector cells *in vitro.* Apparently the tumor-protecting mechanisms are really powerful *in vivo*. This was the apparent conclusion from these experiments.

My colleague, Edward Baral at the Manitoba Cancer Foundation was intrigued by the fact that women with breast cancer will react to Tamoxifen therapy even if the tumor is estrogen receptor negative. (Only one estrogen receptor was known at that time.) He suspected that estrogen might act on the immune system which could explain the results. However, when we tested the effect of tamoxifen on tumor cells we got inhibition whether the tumor was receptor positive or negative. For this reason we postulated the existence of a second estrogen receptor but were not funded for the isolation of this second receptor. The second receptor was described by others within 2 years [14].

Because we were interested in the immune effect of tamoxifen, we did cytotoxicity experiments with tamoxifen treated tumor target cells. We observed enhanced lysis with estradiol or tamoxifen treated tumor target cells in T cell mediated immune lysis [15] [16]. However, when we analysed the mechanism of action we found that both tamoxifen and estradiol stimulated the effector cells and increased the susceptibility of tumor target cells to lysis. This was a dual effect by estradiol and tamoxifen. Tamoxifen apparently increased tumor immunity by this dual action [17]. Similar dual enhancement was observed also by lymphokine activated killer cells [18].

Next we performed animal experiments and established that the anti-estrogens, tamoxifen and toremifen, potentiate the immunotherapy of the P815 murine mastocytoma by cytotoxic T lymphocytes [19] [20]. Experiment with the SL2-5 murine lymphoma and with natural killer cells and tamoxifen or toremifen worked the same way: a therapeutic effect was seen [21].

We published a review paper on the effect of tamoxifen on the immune response [22]. We were asked to write an article in Handbook of Pharmacology and wrote about: physiology and pathophysiology of estrogens [23]. The third review article was about neuroimmunomodulation in cancer [24].

We also studied human breast and lung carcinomas. First we established if these tumors had infiltrating lymphocytes, like in our methycholanthrene induced guinea pig sarcoma. The results were positive. So we digested tumor tissue with collagenase to release all the cells and then separated lymhocytes from tumor cells on Ficoll gradients. So with this method we could obtain autologous (syngeneic) target and effector cells for cytotoxicity experiments. Both breast and lung carcinoma cells were sensitized by anti-estrogens for lysis by autologous tumor infiltrating lymphocytes. These experiments established that our experimental results in animals are valid for cancer patients [25].

At this point 2 review papers were published, one was about the mechanism of anti-estrogens affecting cell mediated tumor lysis [26]. And the other paper announced that we cured mice of cancer with combination immunotherapy [27].

## 3. Discussion

It seems from our results that most, if not all, tumors are infiltrated with killer T cells which are incapacitated *in vivo*, apparently by the enhancing mechanisms of the tumor. However, these cells recover in tissue culture and are capable of killing the cells from the cancer they infiltrated.

The anti-estrogens, tamoxifen and toremifen have a dual effect on cancer immunity; they sensitize the tumor cells for killer cell mediated lyses, and inhibit tumor growth at the same time. For this reason TX and TO were suitable to support the immunotherapy of cancer, as demonstrated with the P815 murine mastocytoma and TK and LAK cells and the SL2-5 murine lymphoma and NK cells. These tumors were killed if TX or TO were used for therapy jointly with the various killer cells used. Killer cells were isolated from human breast carcinomas and lung tumors. TX and TO enhanced the cytotoxicity of autologous killer cells on their corresponding tumor cells. This experiment indicates that our result in animals is also valid for human cancer [28] [29].

A further consideration comes to mind. If one is to design successful therapy protocols, it is not enough to attack the tumor or stimulate the immune system alone. Ideally the tumor should be incapacitated so the immune system can handle the job. A tumor which mutates continuously cannot be destroyed by any agent. Nowadays we have growth factor antagonist agents and immunological adjuvants which could be employed to treat other tumors for therapy. The principle is to inhibit the tumor and stimulate immune function. If this is achieved success will follow.

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