## Control of experimental breast cancer by antioestrogenic therapies

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The dimethylbenz (*a*) anthracene (DMBA)-induced rat mammary carcinoma model has become established as an experimental system for the study of hormone-dependent cancer (Huggins, Grand & Brillantes, 1961). We have used this model to determine the anti-tumour effects of either oestrogen withdrawal (ovariectomy) or antioestrogen therapy (tamoxifen: *trans* 1-(4β dimethylaminoethoxy phenyl) 1,2 diphenylbut-1-ene) before the appearance of palpable tumours i.e.: when the animals have a low tumour burden.

Female Sprague-Dawley rats, 50 days of age, were each given 20 mg DMBA in 2 ml peanut oil p.o. For all experiments, groups contained 20 rats each. In the first experiment, groups were injected daily with tamoxifen (50 μg s.c. in 0.1 ml peanut oil) between 5 and 35 d (A), 15 and 45 d (B), 30 and 60 d (C) and 50 and 80 d (D) after DMBA. This dose of tamoxifen has previously been shown to produce regression of established DMBA-induced tumours (Jordan & Jaspan, 1976). In controls palpable tumours appeared in each rat between 60 and 180 d after DMBA. The rate of tumour appearance in group D was similar to controls whilst tumour appearance in groups A, B and C was delayed for approximately 40 d at the point when all groups had 50% of animals with tumours. We have previously reported that treatment with increasing dose of tamoxifen (0.2, 3, 50 or 800 μg daily) between 30 and 60 d after DMBA results in an initial dose-related delay of tumour production (Jordan & Naylor, 1978).

In the second experiment the antitumour effect of ovariectomy 30 d after DMBA was investigated. No tumours were found in these rats until 150 d after DMBA and tumour appearance was 45% by 200 d compared with the 100% in controls. By contrast, continuous tamoxifen treatment (50 μg daily) between 30 and 200 d after DMBA resulted in tumour production by 200 d in only 10% of animals. Similarly 5% of animals had tumours at 200 d when they were ovariectomized 30 d after DMBA and also treated with tamoxifen between 30 and 130 d after DMBA.

It is concluded that tumour development is only inhibited by the continued presence of the anti-oestrogen. Moreover the delayed tumour development in ovariectomized rats can be further inhibited by anti-oestrogens which suggest that non-ovarian sources of steroids, possibly from the adrenals, can support tumour growth.

These results may have important implications for the use of antioestrogens as an adjuvant therapy after surgery in the treatment of breast cancer. If short courses of antioestrogens only delay tumour recurrence rather than eradicate the metastases then prolonged treatment cycles may need to be considered so that the control of hormone dependent growth is maintained.

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## Studies on the mechanism of action of cyclosporin A

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Cyclosporin A (CS-A) is a small undecapeptide with a mol. wt. of 1203 which has been shown to act selectively on the early phase of lymphoid cell stimulation (Borel & Wiesinger, 1977). Hence, this compound affects most conditions where lymphocytes primarily are involved such as humoral and cell-mediated immunity and chronic inflammatory reactions. Extensive *in vitro* and *in vivo* experimentation in several animal species has revealed that CS-A exerts virtually no effect on any leukocytic or tumour cell except on the immunocompetent T lymphocyte (Borel & Wiesinger, 1977; Borel, Feurer, Magnée & Stähelin, 1977). We have attempted to investigate further the mechanism by which this drug exhibits its highly specific anti-T cell action.

Inhibition of plaque-forming cells in the mouse by CS-A was assessed *in vitro* (Mishell & Dutton, 1966). The $ED_{50}$ was found at approximately 15 ng/ml, a concentration which is also effective in inhibiting proliferation of mitogen stimulated spleen cells (Borel & Wiesinger, 1977). Since it is already known that CS-A did not affect mouse B lymphocytes (Borel *et*

*al.*, 1977), this result demonstrates its action on T helper cells.

In kinetic experiments using cell numbers as the parameter, CS-A (0.1 μg/ml) was added to mouse spleen cell cultures either at the beginning of or 48 h after onset of stimulation by Con A. The drug affected lymphoid cell proliferation only at an early stage of mitogenic triggering and did not interfere with blast cells since no inhibition was observed after addition at 48 h. Inhibition of cell transformation was not due to a cytolytic action of CS-A as no significant differences in cell number nor in percentage of dye exclusion were observed between drug-treated, stimulated and non-treated, unstimulated control cultures.

The reversibility of the inhibitory effect of CS-A was studied using the same *in vitro* system. To a mouse spleen cell culture 0.1 μg/ml of the compound was added for 1 hour. The culture was then washed to remove the drug and Con A added for the next 72 h. The proliferation as measured by cell number was inhibited by 65% of control value compared to 87% in cultures containing the drug during the full incubation period. In another experiment CS-A was washed out after 1 h and the cells were incubated 24 h without drug before Con A was added for the last 72 h. Now the inhibition was only 22% of control value, thereby indicating that the effect of the compound was partly reversible.

Using biochemical assays the content of RNA, DNA and protein from drug-treated and Con A stimulated cultures were shown to be equal to those measured in unstimulated controls. It was further demonstrated that the incorporation of the tritium-labelled precursors (uridine, thymidine and leucine) was strongly inhibited.

These results led us to conclude that CS-A totally inhibits Con A stimulation of resting T cells. It seems to interfere at the very early stage of the cell cycle and does not affect the lymphocyte once it has been triggered.

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## The effect of several immunomodulating agents on a model of humoral and cell mediated immunity in the mouse


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Pharmacological modulation of the humoral (antibody mediated) and cell mediated immune response is complicated by numerous factors that include species, timing and dose of drug administration, antigen, etc. (Heppner & Calabresi, 1976). In attempts to affect selectively the different limbs of the immune system we decided to examine the effects of single doses of a variety of drugs in a combined humoral and cell mediated model of immunity in the mouse. These studies were prompted by the observations that drugs such as cyclophosphamide could enhance the cellular response when administered prior to antigen sensitization (Turk & Poulter, 1972; Turk, Parker & Poulter, 1972; Kerkhaert, van den Berg & Willers, 1974; Lagrange, Mackaness & Miller, 1974).

Male mice (CFLP strain, 30–45 g) were used in groups of 8 for these experiments. Drugs or drug vehicle (5% mulgofen in distilled water) were administered i.p. on the day prior to antigen sensitization. The mice were sensitized s.c. with 0.1 ml of a 0.125% emulsion of methylated bovine serum albumin (MBSA) in Freund's complete adjuvant to elicit a cell mediated immune response and i.p with a suspension of $10^8$ sheep red blood cells to produce a humoral immune response. Seven days after sensitization one hind paw of each animal received 0.05 ml of 0.05% MBSA in saline and the contralateral paw received saline alone. Hind paw thickness was measured 24 h after challenge and the mean change in paw thickness between MBSA and saline injected paws was calculated. Blood samples were obtained from the retro-orbital plexus immediately after the hind paw measurements and the serum from individual mice was used to measure haemagglutinating antibody titres. Vehicle and drug treated groups were compared using the Student's *t*-test and Mann Whitney U-test for the cell mediated immune response and humoral immune response, respectively.

Of the range of drugs examined only the nitrogen mustard type alkylating agents such as cyclophosphamide (200 mg/kg), chlorambucil (30 mg/kg) and melphalan (5 mg/kg) enhanced the cell mediated immune response. A number of other immunomodulating agents such as cycloleucine (300 mg/kg), azathioprine (250 mg/kg), levamisole (30 mg/kg), methotrexate (100 mg/kg), oxisuran (300 mg/mg) and procarbazine (300 mg/kg), and anti-inflammatory drugs such as indomethacin (10 mg/kg) and prednisolone (30 mg/kg) were inactive. Only