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Interleukin-2-induced growth inhibition of prostatic adenocarcinoma (Dunning R3327) in rats*

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Summary. The present study was designed to evaluate the effect of a biological response modifier, interleukin-2 (IL-2), on the growth in rats of Dunning (R3327, androgen sensitive) prostatic adenocarcinoma. IL-2 was given to one group of tumour-bearing rats by subcutaneous infusion (Alzet micro-osmotic pump 2002, 14 days) of 424,286 IU/kg per day during 4 weeks. Another group was sham-operated and served as control. Tumour growth was calculated by weekly measurement of tumour volume. IL-2 treatment caused a significant growth delay without any significant toxicity. Plasma testosterone concentrations were similar in both groups and ventral prostatic weights did not differ. Morphometric analyses of epithelial cells, stroma, luminal compartment in tumour tissue and calculation of the number of intratumoral lymphocytes did not show any differences between the two groups. It is suggested that IL-2 treatment can decrease prostatic tumour growth without apparently affecting the testosterone metabolism. Further studies with special interest on the mechanism of action are justified.

Key words: Interleukin-2 – Prostatic carcinoma – Rat

When prostatic carcinoma growth is established outside the primary site, the long-term prognosis is poor. Current therapeutic approaches, i.e. endocrine therapy, irradiation and chemotherapy, are less successful in improving survival in metastatic cancer.

Furthermore, the efficacy of these therapeutic modalities is limited by their effects on normal tissue. It is also well established that cancer diseases *per se* are afflicted by hampered immunological efficiency. Recent information suggests that patients with prostatic carcinoma also have aberrant immunofunction in the early stages of the disease [2].

Consequently, intensive efforts have been directed towards the use of new modalities in the management of cancer, and interest in recent years has been directed to the development of immunotherapy. The pure biological response modifier interleukin-2 (IL-2) has been shown to cause regression of established growing cancer in animals and humans by strictly immunological mechanisms. Recently, in preliminary observations it has also been suggested that immunological applications can hamper the growth of prostatic carcinoma [8].

To our knowledge, there have to date been no conclusive studies on the effects of IL-2 on prostatic carcinoma. Therefore, it is of interest to report that IL-2 administration alone caused a significant reduction in the growth of the Dunning (R3327, androgen sensitive) prostatic carcinoma in rats.

Materials and methods

Animals

A 1 mm^3 core of prostatic adenocarcinoma tissue (Dunning R3327) was implanted bilaterally into each flank of 6–10-week-old male offspring of Copenhagen × Fischer F1 rats at the Department of Physiology, University of Umeå. The tumours were originally obtained from Dr. N. H. Altman (Organ System Program of the National Cancer Institute, USA) and kindly supplied to us by Dr. P.-I. Kristensson (Leo AB, Helsingborg, Sweden). The animals were housed in a controlled environment (12 h light/12 h dark) with pellets and water freely available. Approximately 3 months after implantation, when the weight of the rats had reached 460 (430–480) g and tumours had a median volume of 149 mm^3, ten rats were chosen at random for IL-2 treatment and ten were used as controls.

IL-2 administration

IL-2 was continuously delivered as a subcutaneous infusion using an Alzet micro-osmotic pump (model 2002) during 4 weeks of treat-

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Table. 1. Body weight, kidney weight and ventral prostate weight of rats after 4 weeks of interleukin-2 (IL-2) treatment and controls

Parameter	Controls ($n = 10$)	IL-2 ($n = 10$)	
Rat weight	455 ± 17 (460 ± 15)	458 ± 14 (466 ± 36)	NS
Kidney weight	1.11 ± 0.03	1.12 ± 0.02	NS
Prostate weight	0.260 ± 0.02	0.295 ± 0.02	NS

Data are given as mean ± SEM. Weights before start of IL-2 treatment are given in parentheses.
Mann Whitney U-test: NS, not significant ($P > 0.1$)

Table 2. Volume densities of different compartments of Dunning R3327 prostatic adenocarcinoma and IL-2 treated animals

Parameter	Controls ($n = 10$)	IL-2 ($n = 10$)
Epithelial cells	51.2 ± 3.1	55.2 ± 3.7
Lumina	16.1 ± 3.5	14.7 ± 2.9
Stroma	30.2 ± 2.9	30.1 ± 3.7

Values are expressed as percentage of total volumes (mean ± SEM)

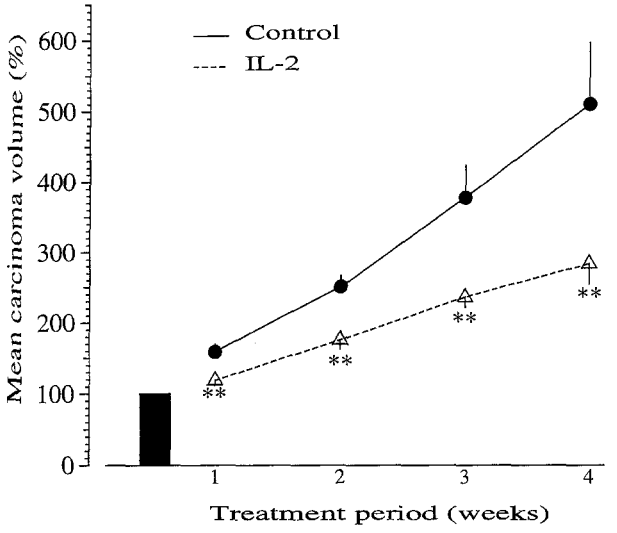

Fig. 1. Median tumour volumes in the control and IL-2 treated rats during the investigation period (28 days). The volumes were estimated by using the formula of an ellipsoidal mass and the tumour volume index was equal to tumour volume/tumour volume at start of treatment × 100%

ment (Alza Corporation, Palo Alto Calif.). This model makes it possible to deliver a steady concentration of drug (0.5 µl/h for 14 days). The pumps were filled with IL-2 solution in a concentration of 1,800,000 IU. in 200 µl, which corresponds to a daily dose of 424,286 IU/kg. After 14 days new pumps with fresh solution were implanted by the same method. The control animals were sham operated and treated in parallel.

Tumour growth

From day 1 of treatment three perpendicular tumour diameters were measured weekly with micro-calipers while the rats were under light ether anaesthesia. Tumour volumes were estimated by using the formula of an ellipsoidal mass [4]; the tumour volume index was equal to tumour volume/tumour volume at start of treatment × 100%.

Morphological and morphometric analysis

Three randomly chosen samples from each tumour were fixed in Bouin's solution and embedded in metacrylate plastic (Histo-Resin, LKB, Stockholm, Sweden). The tissue was cut into 2-µm-thick sections and stained with haemotoxylin- and eosin. The morphology and the volume densities of the tumour epithelium, stroma and acinar lumina were determined at × 250 magnification using a square lattice mounted in the eyepiece of a light microscope. The number of test points lying over each of these tissue compartments was counted using the method of Weibel [10].

Chemicals and assay

IL-2 was kindly supplied by Farmos AB, Stockholm, Sweden and Euro Cetus, Amsterdam, The Netherlands. Testosterone concentration in plasma was analyzed by radioimmunoassay [1]; the intra-assay coefficient of variation of the method was 9% [1].

Results

General condition

The animals tolerated the IL-2 treatment well, without any significant signs of toxicity. No significant changes in body weight, kidney or prostate weight were seen (Table 1).

Tumour growth

Treatment of rats with IL-2 caused a significant and continuous reduction in tumour growth during the period of investigation (28 days) compared to the tumours in control animals (Fig. 1). When the tumour weights were correlated to calculated volumes, the coefficient of correlation was 0.91.

Morphological analysis

The Dunning tumours were fairly small and did not contain necrotic areas. Treatment with IL-2 did not influence the general morphological appearance of the tumour. This observation was supported by the morphometrical analysis (Table 2). The Dunning tumour stroma is composed of fibroblasts, smooth muscle cells, large stromal cells, blood vessels and occasional macrophages and mast cells. The stroma contained extremely few lymphocytes without any obvious differences between the

two groups. IL-2 treatment did not induce any apparent increase in lymphocyte numbers inside the tumour parenchyma or in the interface between the tumour and adjacent tissue. A discrete inflammatory response was observed in two of the ten intact tumours examined and in three of the ten tumours treated with IL-2.

Testosterone

There were no significant changes in the plasma level of testosterone in the IL-2 treated group (1.61 ± 0.75 ng/ml) compared to the controls (1.56 ± 0.29 ng/ml) (mean ± SEM). This was in accordance with the finding that the prostate weights were unaltered in IL-2 treated rats as compared to controls (Table 1).

Discussion

The present study has shown that IL-2 significantly affects the growth of the Dunning (R 3327) prostatic carcinoma in rats. It is in accordance with earlier observations that a direct and specific manipulation of the immune system can mediate regression of different kinds of manifest cancer in experimental models as well as in humans. Other preliminary data on the Dunning prostatic adenocarcinoma (AT-3 and Mat-Lu-Ly) indicate that LAK cells + IL-2 as well as the combination of TIL cells, cyclophosphamide and IL-2 prevent pulmonary metastasis and retard tumour growth [2–4].

Despite the clear difference in tumour volume (Fig. 1) the histological appearance of control and IL-2 rats was similar although the therapeutic effects of IL-2 on experimental tumours are considered to be mediated by local accumulation of immune cells. In the study we did not observe any apparent lymphocytes accumulation inside or around the tumours as a result of IL-2 treatment. It should, however, be noted that we only studied the morphology after 4 weeks of continuous treatment and we can therefore not exclude the possibility of an immune response in the tumour earlier in the treatment period. No increase in the number of leucocytes was found within tumour tissue, although in some rats increased numbers of leucocytes were found in the peritumoral vasculature. It has previously been shown that IL-2 can affect tumour blood flow and vascular permeability in other systems [6, 7]; this deserves further attention in order to in the evaluation of the plausible mechanisms of action.

IL-2 treatment has profound effects on several hormonal systems [6]. It has also been established that this Dunning tumour model is basically dependent on the trophic effects of androgen (J. E. Damber et al., manuscript in preparation) as well as the hormone requirements for the maintenance of normal prostate weight. The unchanged levels of testosterone in IL-2-treated animals compared to controls indicated that the antitumoral effects did not involve hormone-dependent processes, i.e. androgen blockade. This was further substantiated by the fact that the weights of the prostatic glands did not differ between the two groups and prostate weight is dependent on the influence of testosterone, which has also been shown in the animals used.

Although the growth retardation following IL-2 treatment in the present model seems to be of similar magnitude as is the case following castration, further studies are needed to evaluate IL-2 in relation to other hormone treatment modalities. Interestingly, 6-methylene progesterone, a new 5αreductase blocker, induces suppression of Dunning tumour growth [9] and normal rat ventral prostate [5] in a similar magnitude as IL-2, without changing morphology. This indicates that factor other than testosterone are important for tumour growth and possibly indicate that IL-2 treatment affects local factors of importance.

In conclusion, the present study shows that growth of the Dunning prostatic carcinoma can be retarded by treatment with IL-2, apparently without involvement of androgen-dependent mechanisms.

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