

GENE THERAPY BY *IN VIVO* ELECTRO GENE INJECTION

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Studies have suggested that the host's immune system is activated after tumour treatment with electropermeabilization. In a study by Mir *et al.*, (1991), immunogenic fibrosarcoma was treated with ECT in immunocompetent syngeneic mice and immunodeficient (nude) mice. The treatment was effective on the immunocompetent mice but only a temporary delay in tumour growth was observed in the nude mice group. A stimulated immune response, through increased monocyte and T-lymphocyte activity was confirmed by Serca *et al.*, (1996) in a similar study, but using cisplatin instead of bleomycin.

Paper VI describes the presence of macrophages and T-lymphocytes specifically in the tumour vicinity after ECT. Two weeks after ECT treatment of colorectal tumours in the liver, tumours were excised, sectioned and stained for CD4, CD8 positive lymphocytes and macrophages. The presence of macrophages and lymphocytes were evaluated in viable tumour. The results showed overall low levels of CD4 positive lymphocytes and ED2 positive macrophages. The largest amounts of CD8 positive lymphocytes were found in viable tumour treated with electric pulses (EP) and bleomycin and the lowest amounts in tumours treated with bleomycin. The largest amounts of ED1 positive macrophages were found in tumours treated with EP and EP+bleomycin. The ECT treatment also seemed to have antimetastatic effects. Surgical excision of the tumour-bearing lobe, as a treatment, fails to prevent development of intraabdominal tumour spread. In a study by Möller *et al.* (1998), using the exact same strain and tumour as in this study, intraabdominal spread was found in 5 of 8 animals, 12 days after resection of the tumour-bearing liver lobe. The tumour is aggressive and 12 days after sham treatments, approximately 75% of the animals show intraperitoneal spread. After ECT, not one animal out of 13 treated showed signs of tumour spread 14 days after treatment. These results strongly suggest that ECT stimulates an activation of the host's immune system against the tumour and prevents tumour spreading

Electropermeabilization and immunotherapy

The combination of electrochemotherapy and immunotherapy has been described in studies by Mir and colleagues. Systemic, antimetastatic immune responses were achieved by delivering histoincompatible cells secreting interleukin-2 (IL-2) in combination with ECT. The tumours investigated were 3LL Lewis lung carcinoma in mice (Mir *et al.*, 1995; Orłowski *et al.*, 1998) and VX2 tumours transplanted in rabbit liver (Ramirez *et al.*, 1998). Histoincompatible xenogeneic (interspecies genetic disparity) cells and allogeneic (intraspecies genetic disparity) cells were engineered to secrete IL-2. These IL-2-secreting cells were injected intratumourally or in the peritumoural oedema after ECT.

The addition of immunotherapy to the ECT treatment of rabbit liver tumours increased the cure rate from 30 to 40% and, more important it had a clear antimetastatic effect on tumour spread in liver and lung. Injection of IL-2-producing cells in combination with ECT, and only this treatment, performed on the 3LL tumours in mice, led to cures of also the untreated contralaterally implanted tumours. This systemic antitumour effect also resulted in immunity against the tumour and complete protection against further tumour cell inoculation. The study by Orłowski *et al.*, (1998), using the same strain and tumour, showed a significant decrease in the number of lung metastases after ECT and injection of IL-2-producing cells.

Immunotherapy with EP in combination with syngeneic IL-18 and IFN- γ secreting tumour cells

In an attempt to achieve an immunoreaction against implanted brain tumours, rats with N29 glioma tumours were delivered with electric pulses followed by injections of IL-18 and IFN- γ secreting cells (Engström *et al.*, unpublished data).

Tumours were inoculated subcutaneously on both thighs of female F-344 syngeneic rats. The left tumour was treated once with 16 pulses of 1400 V/cm, 1.0 ms duration (time constant).

No anticancer drugs were given at any time. The following day and then once weekly for three weeks, the animals were given intraperitoneal injections of irradiated, modified N29 tumour cells, secreting either interleukin-18 (IL-18) or interferon- γ (IFN- γ).

The results were evaluated by measuring the growth of the *untreated contralateral* tumours. There was no difference in contralateral tumour growth between animals given no treatment, electric pulses only, IFN- γ secreting cells only or IL-18 secreting cells only. A significantly inhibited growth rate was observed, in animals given EP with IFN- γ secreting cells and in animals given EP with IL-18 secreting cells but in no other combination. This treatment resulted in a prolonged survival (the time for the contralateral tumour to reach the predetermined limit volume), by 50%. These results show that a systemic response of the host's immune system can be achieved against the tumour, using *syngeneic* tumour cells, and that this may be an important step towards effective tumour immunotherapy.

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