

Editorial

Gene Therapy of Cancer by Vaccines Carrying Inserted Immunostimulatory Genes

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Therapeutic strategies based on the insertion of cytokine or other immunostimulatory genes into the genome of tumour cells followed by vaccination with the resulting genetically modified, cytokine-producing, irradiated vaccines represent a new potential prospect for the treatment of cancer patients. The vaccines carrying inserted immunostimulatory genes were, for the first time, designed and successfully utilized in 1988 (Bubeník et al., 1988). Fibroblastoid RAT-1 cells were *in vitro* transfected with interleukin (IL)-2 cDNA using a retroviral vector. The genetically engineered RAT-1 cells constitutively producing IL-2 were capable of inhibiting human tumour xenografts transplanted in congenitally athymic (nu/nu) mice (Bubeník et al., 1988) as well as Rous sarcoma virus-induced tumours transplanted in syngeneic Lewis rats (Bubeník 1989). As compared with repeated systemic high-dose IL-2 administration required for inhibition of the tumours, which frequently produces severe adverse effects, a single injection of the IL-2-producing RAT-1 cells could secure a long-lasting therapeutic IL-2 concentration in the tumour vicinity with a negligible IL-2 concentration in the peripheral blood and, therefore, with no adverse effects. These first findings were later confirmed and extended by other laboratories using various murine tumours, such as fibrosarcoma (Gansbacher et al., 1990), colon carcinoma (Fearon et al., 1990), mastocytoma (Ley et al., 1991), as well as plasmacytoma (Bubeník et al., 1991) and leukaemia (Bubeník et al. 1995). Insertion of some other immunostimulatory cytokine genes, IL-4, IL-6, IL-7, tumour necrosis factor (TNF)- α or interferon (IFN)- γ into tumour, dendritic or stromal cells also pro-

vided cellular vaccines with similar tumour inhibitory effects (for a review, see Bubeník et al., 1996a; 2001). It has been found that the defence reaction initiated by the gene therapy continues for some time after administration of the irradiated vaccine or after silencing/segregation of the inserted genes, in other words, for a period sufficient not only for the rejection of the tumour, but also for the development of the “protective” immunological memory. The immune reaction induced by the gene therapy was able to destroy not only the genetically manipulated cells carrying the inserted molecules, but also the original, parental tumour or other cells (for a review, see Bubeník 1996b).

In experimental tumour systems the most encouraging results have been obtained with treatment of small tumours, distant micrometastases, and residual tumour disease after surgery or chemotherapy (Bubeník et al., 1999; 2003; Mikyšková et al., 2001; 2004; Indrová et al., 2003; Bubeník and Šimová 2005). To increase the efficacy of the genetically modified tumour vaccines, various combinations of cytokine-secreting vaccines with other cytokines or other therapeutic modalities were examined (for a review, see Bubeník 1996a).

Taken together, the preclinical studies have demonstrated that cytokine genes can be successfully inserted into the genome of tumour cells, that the genetically engineered tumour cells may secrete therapeutic levels of cytokines, and that vaccination with the genetically modified, cytokine-secreting tumour or other cells can substantially inhibit tumour growth in a variety of experimental model systems and can induce immunological memory.

Safety studies in preclinical tumour systems indicated that inactivated rather than live cell vaccines should prospectively be used for treatment of cancer patients although the insertion of cytokine genes substantially decreases the tumorigenicity of the parental tumour cells. Consequently, γ -irradiation of the IL-2- or IFN- γ -producing tumour vaccines with doses that do not eliminate cytokine secretion but eliminate tumorigenicity has been used in human melanoma and renal carcinoma cell lines. In pilot experiments these cell lines continued to secrete cytokines in sufficient quantities for several weeks after irradiation with 50–100 Gy (Gansbacher et al., 1992; Gastl et al., 1992). A wide range of cancers was exam-

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Abbreviations: CEA – carcinoembryonic antigen, GM-CSF – granulocyte-macrophage colony-stimulating factor, IFN – interferon, IL – interleukin, TNF – tumour necrosis factor.

ined in clinical phase I–II trials (Foa et al., 1994; Sobol et al., 1995; Nemunaitis 2003; Cunningham et al., 2005; Kikuchi, 2006; Nemunaitis et al., 2006), including glioblastomas, non-small-cell lung carcinomas, colon carcinomas and melanomas. Recently, numerous phase II–III clinical trials with these carcinomas and pancreatic cancer, prostate cancer, lymphomas and renal cell carcinomas are in progress. Autologous tumour cells, allogeneic cells as well as recombinant replication incompetent vaccinia, fowlpox virus or adenoviruses engineered to produce CEA, mucin MUC-1 antigen, GM-CSF, CD40 ligand, IL-2, CD80 or TNF- α are being carefully tested. Mixed results have been produced, highlighting both the potential for the gene therapy and the areas that still need to be perfected before the genetically engineered cancer vaccines can become part of standard cancer treatment (for a review, see Cross and Burmester, 2006). Some of the studies are being performed under particular auspices and support of EU Life Sciences Programme. One example is the European Network of Excellence Clinigene Project (No. 018933, EU-FP6, NoE), which was approved with the final aim to produce, at the end of this decade, first clinically utilizable GMP products for gene therapy including cellular vaccines for treatment of cancer patients. However, also in this instance the available vectors, vaccination protocols and cellular vaccines have first to be optimized. At present, the optimal combinations of cytokines with cellular vaccines, chemotherapeutics and other therapeutic modalities are being found. The results of the phase II – III clinical trials (review in Cross and Burmester, 2006) as well as other results (EU FP6, FP7) have to be carefully evaluated before the definitive conclusions regarding the actual therapeutic potency of this novel and promising strategy for the management of cancer patients can be drawn.

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