Interleukin-2 Therapy of Cancer

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Abstract. The aim of this review is to evaluate the results of IL-2 therapy of cancer two decades after the first experiments and to discuss whether and which results of local, systemic and adjuvant IL-2 therapy in preclinical models can be translated into clinics. The attention is also focused on the development and utilization of the IL-2 gene-modified tumour vaccines for therapeutic purposes. The prospects and limitations of both, IL-2 therapy and IL-2 gene therapy are discussed.

In 1976, Morgan, Ruscetti and Gallo described a new glycoprotein capable of supporting selective *in vitro* growth of T lymphocytes from normal human bone marrow. A similar glycoprotein was also found in mice. The glycoprotein was designated as T-cell growth factor or interleukin-2 (IL-2). Human IL-2 is a glycosylated polypeptide with a molecular mass of 15.5 kDa; it is produced in an autocrine and paracrine manner mainly by the activated cells of T and NK lineages. Interleukin-2 transmits activating and/or mitogenic signals to cells carrying functional high-affinity IL-2 receptors; these cells also belong primarily to T and NK cell lineages (Table 1).

In 1983, we found that peritumoral injections of rat lymphoid IL-2 could suppress or markedly inhibit the growth of methylcholanthrene-induced sarcomas in syngeneic mice. An equally effective inhibition of murine sarcoma transplants in syngeneic recipients could be obtained with crude lymphoid rat IL-2, with purified IL-2 of murine lymphoid origin, and with molecularly homogenous human recombinant IL-2 (Bubeník et al., 1983, 1985, 1986). The tumour-inhibitory effects of local IL-2 administration were repeated and confirmed in various experimental tumour systems (for a review, see Bubeník et al., 1992; Bubeník, 1994).

In 1984, the first evidence that local administration of IL-2 can inhibit growth of human tumours was obtained by Pizza and collaborators, who described tumour regressions after intralesional injections of IL-2 in bladder cancer. One year later, Rosenberg and collaborators (1985a) succeeded in inhibiting growth of metastatic experimental tumours by systemic administration of IL-2 and brought the first evidence that systemic administration of autologous IL-2-activated killer cells (LAK) and recombinant IL-2 is efficient in patients with metastatic cancer (Table 1). Repeated cycles of IL-2 administration were required for the systemic tumour-inhibitory effects. The efficient doses of IL-2 for the systemic treatment of human cancer had to be very high; they were toxic and induced adverse effects, which occassionally led even to the death of the treated patients. To overcome these problems we have proposed to insert the cloned IL-2 gene into tumour cells and to use such genetically modified, irradiated tumour vaccines for peritumoral administration as a local source of IL-2. In the 1988-1989 period it was demonstrated that a single injection of the genetically modified IL-2-producing vaccine can cure mice bearing progressively growing transplants of xenogeneic human carcinomas as well as rats bearing syngeneic Rous sarcoma virus-induced sarcomas and mice carrying syngeneic plasmacytomas (Bubeník et al., 1988, 1990; Bubeník, 1989). The experiments with genetically modified tumour vaccines were repeated by many other groups; various cytokines and different experimental tumour models were utilized (for a review see Bubeník, 1993a,b, 1994, 1996a,b). The resulting new modality of cancer treatment was designated as cytokine gene therapy of cancer. The IL-2 gene has later been inserted into human tumour-infiltrating lymphocytes (TIL) and human tumour cells and the resulting genetically modified vaccines constitutively producing IL-2 were finally used even for clinical trials (Table 1, section 3b).

Tumour-inhibitory effects of IL-2 in preclinical model systems

Local and systemic IL-2 immunotherapy

Interleukin-2 provides the activating and mitogenic signal (Table 1) required for the differentiation and expansion of the principal tumour defence effectors, T and NK lymphocytes; it has no direct cytolytic or cytostatic effects on

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Abbreviations: CMRTD – minimal residual tumour disease after chemotherapy, CTL – cytotoxic T lymphocytes, IFN – interferon, IL-2 – interleukin 2, LAK cells – lymphokine-activated killer cells, NK cells – natural killer cells, PBL – peripheral blood lymphocytes, SMRTD – surgical minimal residual tumour disease, TIL – tumour-infiltrating lymphocytes.

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Table 1. IL-2 therapy and IL-2 gene therapy of cancer: how one finding has led to another

Event	Author
Discovery of IL-2	Morgan et al. (1976)
Mitogenic IL-2 signal and long-term cultures of tumour-specific CTL	Gillis and Smith (1977)
Activating IL-2 signal (LAK cells)	Grimm et al. (1982)
Local administration of IL-2 inhibits tumour growth in preclinical models	Bubeník et al. (1983)
Local administration of IL-2 inhibits growth of human tumours	Pizza et al. (1984)
Systemic administration of IL-2 inhibits growth of metastatic experimental tumours	Rosenberg et al. (1985a)
Utilization of recombinant IL-2 for clinical trials in patients with generalized tumours	Rosenberg et al. (1985b)
Local IL-2 gene therapy inhibits tumour growth in preclinical models	Bubeník et al. (1988)
Utilization of TIL or tumour cells carrying an inserted IL-2 gene for the first clinical trials	for reviews, see Anderson (1992); Foa et al. (1992)

tumour cells. The exogenous IL-2 administration was efficient in a broad spectrum of experimental tumours, including sarcomas, carcinomas, haemoblastoses, melanomas and hepatomas (Bubeník et al., 1983, 1985, 1986; Kedar et al., 1985; Lafrenière and Rosenberg, 1985; Rosenberg et al., 1985a; Maekawa et al., 1986; Thompson et al., 1986; Silagi and Schaefer, 1986; Hinuma et al., 1987; Vaage, 1987, 1988; Vaage et al., 1987; Rutten et al., 1989; Liu et al., 1990; Maas et al., 1991; Konno et al., 1991; Balemans et al., 1994). Selected examples of the local and systemic IL-2 effects are illustrated in detail in Tables 2 and 3.

Interleukin-2 was particularly effective when injected repeately during the early period of tumour growth; it was also highly efficient in the individuals carrying tumour residua after surgery (surgical minimal residual tumour disease (SMRTD)) or after chemotherapy (CMRTD), but not in the animals with large or generalized neoplasms.

In the first report describing IL-2 treatment of SMRTD (Vlk et al., 1998) we found that IL-2 or IL-2-producing vaccines had to be administered 2–5 days after surgery at the site of the surgery. The relatively narrow time window for the successful IL-2 treatment of SMRTD was repeatedly confirmed in various murine tumour models (Bubeník et al., 1999; Mikyšková et al., 2001, 2004; Bubeník et al., 2003).

Peritumoral IL-2 administration could also substantially inhibit growth of tumour residua after chemotherapy with cyclophosphamide or ifosfamide derivatives (Bubeník et al., 1995; Indrová et al., 2003). Interestingly, peritumoral administration of IL-2 in the vicinity of s.c. transplanted tumours and in mice with SMRTD and CMRTD inhibited not only the growth of the s.c. primary tumours or tumour recurrences, but also their lung metastases (Mikyšková et al. 2001, 2004; Indrová et al., 2003).

Therapy with IL-2 gene-modified tumour vaccines

Exogenous IL-2 has a very short half-life after *in vivo* administration. Therefore, unphysiological, extremely high, subtoxic and repeated IL-2 doses were required to maintain the efficient IL-2 level in the circulating blood during the systemic treatment of experimental neoplasms. We have proposed an alternative mode of IL-2 administration, which could substantially

Table 2. Local and regional IL-2 immunotherapy of cancer in preclinical models

Interleukin-2	Dose (units/mouse)[a]	Tumour	Complete response (%)	Reference
rat lymphoid	2.0×10^1	MC-induced murine sarcomas	80	Bubeník et al. (1983)
murine lymphoid	1.0×10^1	MC-induced murine sarcomas	25	Bubeník et al. (1985)
human recombinant	3.0×10^3	MC-induced murine sarcomas	62	Bubeník et al. (1986)
human recombinant	3.5×10^5- 5.0×10^5	murine myeloma	50-100	Maekawa et al. (1986)
human recombinant	2.5×10^5	murine myeloma	87-100	Silagi and Schaefer (1986)
human recombinant	3.6×10^3-1.2×10^4	murine mammary carcinomas	10-63	Vaage (1987)
human recombinant	6.0×10^4-1.2×10^6	murine mammary carcinomas	0-50	Vaage (1988)
human recombinant	1.8×10^4	murine mammary carcinomas	11-37	Vaage et al. (1987)

[a]The definition of IL-2 units is different in different papers.

Table 3. Systemic IL-2 immunotherapy of cancer in preclinical models

Tumour	Dose (units/mouse)	Response	Reference
murine chemically induced sarcomas and melanoma	1.5×10^5 - 3.0×10^5	regression of transplanted tumours and pulmonary metastases	Rosenberg et al. (1985a)
murine chemically induced sarcoma	1.5×10^4 - 7.5×10^4 (+ LAK)	regression of hepatic metastases (IL-2 + LAK more efficient than IL-2)	Lafreniere and Rosenberg (1985)
murine pulmonary and mammary carcinomas	2×10^2 (+ chemotherapy + CTL)	regression of transplanted tumours	Kedar et al. (1985)
murine erythroleukaemia	4.8×10^4	complete remission in 50% of mice	Thompson et al. (1986)

reduce the IL-2 toxicity and its adverse effects. In the 1988 period we had inserted a cloned IL-2 gene into tumour cells, and used such genetically modified tumour vaccine, producing IL-2 in a low, physiological, and long-lasting quantity for peritumoral injection (Bubeník et al., 1988; Bubeník, 1989). Single administration of irradiated, IL-2 gene-modified cellular vaccine secured the therapeutic level of IL-2 in the tumour vicinity and low IL-2 level in the circulation. Since the high IL-2 level in the circulating blood was responsible for the adverse effects during the systemic IL-2 administration, such as the capillary leakage syndrome, hepatotoxicity and nephrotoxicity, a single peritumoral injection of the genetically modified, low-level IL-2-producing vaccine could avoid the adverse effects. To demonstrate the tumour-inhibitory effect of the cytokine gene-modified vaccines, three different experimental designs were employed during the early period of the vaccine development (Table 4). In some experimental systems, prophylactic immunization with a genetically modified tumour vaccine followed by a subsequent challenge with the parental tumour was utilized. In other models, co-injection of the genetically modified and parental tumour cells was used. A third group of laboratories employed a clinically relevant therapeutic design in which a temporary growth of the parental tumour was followed by vaccination with the genetically modified, cytokine-producing tumour vaccine (for a review, see Bubeník, 1996a,b). The results obtained in the experiments with the "prophylactic" or "co-injection" design (Table 4) revealed that the effect of some vaccines was weak and did not allow for the real therapeutic utilization of the vaccines (Fearon et al., 1990; Gansbacher et al., 1990; Ley et al., 1991; Belldegrun et al., 1993). The "prophylactic" and "co-injection" experiments, however, could serve for the demonstration of the immunizing capacity of the genetically modified vaccines, for the detection of the mechanisms of the induced immunity, for demonstration that the immune reaction elicited by preimmunization with the cytokine-secreting tumour cells can cope not only with the cytokine-secreting cells, but also with the parental cells. These systems also served for demonstration of the long-lasting specific immunological memory directed against parental cells, induced by the rejection of the genetically modified, cytokine-secreting cells. However, the conclusive results with regard to the treatment of cancer were only obtained in the experiments using the "therapeutic" design (Bubeník et al., 1988; Bubeník, 1989; Bubeník et al., 1990; Porgador et al., 1992; Connor et al., 1993; Vieweg et al., 1994). These results have shown significant therapeutic effects with the IL-2 gene-modified, IL-2-producing live tumour vaccines already in the first experimental

Table 4. Tumour-inhibitory effects of IL-2-producing vaccines

Tumour type	Design of the experiment	Reference
(a) Live vaccines		
human cervical carcinoma	therapeutic (nu/nu mice)	Bubeník et al. (1988)
rat sarcoma	therapeutic	Bubeník (1989)
murine plasmacytoma	therapeutic	Bubeník et al. (1990)
murine fibrosarcoma	co-injection	Gansbacher et al. (1990)
murine colon carcinoma	prophylactic	Fearon et al. (1990)
murine mastocytoma	prophylactic/co-injection	Ley et al. (1991)
(b) Irradiated vaccines		
murine lung carcinoma	therapeutic	Porgador et al. (1992)
murine bladder carcinoma	therapeutic	Connor et al. (1993)
rat prostate carcinoma	therapeutic	Vieweg et al. (1994)
murine leukaemia	therapeutic	Bubeník et al. (1995a,b)
human renal carcinoma	co-injection	Belldegrun et al. (1993)

systems utilized, in human cervical carcinoma xenografts growing progressively in congenitally athymic (nu/nu) mice (Bubeník et al., 1988), in Rous sarcoma virus-induced rat sarcomas transplanted in syngeneic inbred Lewis rats (Bubeník, 1989) and in murine plasmacytomas transplanted in syngeneic BALB/c mice (Bubeník et al., 1990). The genetically modified therapeutic tumour vaccines were capable of substantially inhibiting tumour growth, irrespective of the fact that the parental, genetically unmodified tumour vaccines were not efficient, also in murine lung and bladder carcinomas, rat prostate carcinoma, and murine leukaemia (Table 4). Similarly to the experiments with the recombinant IL-2, also the experiments with IL-2 gene-modified tumour vaccines could be performed in clinically relevant settings, SMRTD and CMRTD (Vlk et al., 1998; Bubeník et al., 1999, 2003; Mikyšková et al., 2001, 2004; Indrová et al., 2003). Surprisingly, the irradiated, IL-2-producing tumour vaccines were found to be more efficient than the live vaccines (Šímová et al., 1998, 2000).

Therapeutic effects of IL-2 in cancer patients

Local and systemic IL-2 immunotherapy

Based on the preclinical model experiments, the first clinical trials using local IL-2 administration were performed. Pizza et al. (1984) used the transurethral administration of IL-2 into the immediate vicinity of urinary bladder carcinomas. Repeated injections of IL-2 under cystoscopic control resulted in a complete regression of the tumour in three out of ten patients and in partial regression in another three patients. The regressions were estimated by the biopsy and with the help of cystoscopic examination; complete regressions lasted over the whole observation period, 2, 4, and 7 months.

The first pioneer experiments performed with IL-2 in humans were repeated with other tumour types (Table 5). Yasumoto et al. (1987) injected IL-2 into the pleural exudate of patients suffering from bronchogenic carcinomas; in nine of the 11 patients treated in this way, exudates were resorbed between the 4th and the 10th day after starting the therapy and tumour cells disappeared in the pleural fluid. Yoshida et al. (1988) observed in six out of 23 patients with recurrent malignant gliomas, treated by repeated injections of IL-2 + LAK cells directly into cavities of tumours, regressions of the gliomas. Those were patients non-responding to conventional radiochemotherapy and surgical therapy; in three out of the six successfully treated patients the injection of IL-2 induced a remission lasting more than 6 months. A group of Forni (Cortesina et al., 1988; Forni et al., 1988) used IL-2 for the therapy of patients with recurrent spinocellular carcinomas of the head and neck. Interleukin-2 was injected into the region of lymphatic vessels at the site of the insertion of the sternocleidomastoid muscle to the processus mastoideus; IL-2 was administered repeatedly, ipsilaterally to the location of the tumour process. Tumours of six out of ten patients responded to the therapy with IL-2; of the six tumours, three responded by complete regression and those of another three patients by partial regression. The positive response to the therapy was observed only in patients without a radical excision of cervical lymphatic nodes. The remission was present throughout the time of the observation, i.e. 5 to 8 months (Cortesina et al., 1988). However, when these successful head and neck cancer treatment results were re-evaluated after a longer period, and repeated by other groups, the results were substantially less optimistic (Rivoltini et al., 1990; Squadrelli-Saraceno et al., 1990; Mattijjsen et al., 1991; Whiteside et al., 1993). In addition to the data reported by Pizza et al. (1984), Huland and Huland (1989) described complete, histologically confirmed remission lasting more than 6 months in one out of five patients with urinary bladder carcinoma after continuous IL-2 perfusion of the bladder performed for 5 days. The first experiments exemplified in Table 5 were extended and repeated by other groups and their results were not substantially different (Radny et al. 2003; for a review see Bubeník, 1990; Bubeník et al., 2000; van Herpen and De Mulder, 2000). A novel way of regional IL-2 administration was an inhalation therapy, proposed by Huland et al. (1992). High-dose IL-2 inhalation therapy with very low toxicity and low level of adverse

Table 5. Local and regional IL-2 administration in cancer patients – selected examples from the early experiments

Interleukin-2	Dose (units/patient)	Tumour	Response (%) Complete /partial	Reference
gibbon lymphoid	$1.5 \times 10^2 – 4.0 \times 10^3$	urinary bladder carcinomas	30/30	Pizza et al. (1984)
human recombinant	$1.0 \times 10^4 – 2.8 \times 10^4$	lung carcinomas	0/82	Yasumoto et al. (1987)
human recombinant	$8.0 \times 10^2 – 5.4 \times 10^3$	malignant gliomas	13/13	Yoshida et al. (1988)
human lymphoid	2.0×10^3	squamous cell carcinomas	29/29	Forni et al. (1988)
human lymphoid	2.0×10^3	squamous cell carcinomas	30/30	Cortesina et al. (1988)
human lymphoid	continuous perfusion 1.5×10^6/week	urinary bladder carcinomas	20/n.v.	Huland and Huland (1989)

n.v., not verified because of incomplete transurethral resection

effects has been used in more than 200 patients with metastatic renal cell carcinomas, melanomas, breast and ovarian carcinomas (Huland et al., 1992, 2000; Enk et al., 2000). When the results obtained during 6 years of inhalatory IL-2 therapy in 116 metastatic renal cell carcinoma patients were evaluated, it was concluded that the progressive pulmonary metastases responded dramatically in 15% of patients for a median of 15.5 months and were stabilized in 55% of patients for a median of 6.6 months (Huland et al., 1997).

In contrast to the above local tumours and local IL-2 therapy, tumour diseases in most organ locations and stages of the progression are seldom local diseases. As systemic diseases, they also call for a systemic therapy. The first communications describing a therapeutic effect of the IL-2 systemic administration in patients with generalized tumours resistant to conventional therapy were published by Rosenberg et al. (1985b, 1987, 1988). It was found that in some patients IL-2 induced partial as well as complete regressions of primary tumours and their metastases (Table 6). These communications stimulated further research activities at many clinical institutions in the United States and in Europe (West et al., 1987; Sosman et al., 1988; Lindenmann et al., 1989). Although the percentage of tumours responding by a complete or partial regression to the therapy with IL-2 was rather low, it is necessary to realize that those were generalized tumours, responding no more to conventional therapy by both cytostatics and radiation and that research of the combination of the administration of IL-2 with conventional pharmaceuticals (Lindenmann et al., 1989) and with other immunomodulators, as well as the research of the administration of IL-2 in less advanced stages of the tumour growth, were only at the stage of their beginning.

Nevertheless, the results of five-year observation of the most extensive set of patients treated by systemic administration of IL-2 and summarized in 1988 demonstrated that particularly in two types of malignant tumours, renal cell carcinoma and melanoma, a considerable percentage of complete as well as partial regressions of generalized tumours can be achieved; the duration of these regressions was on average 6 months, in certain patients even 20 to 31 months (Rosenberg, 1988).

Comparison of the percentage of regressing tumours after administering IL-2 itself and IL-2 + LAK cells (Table 6) has demonstrated that the combined therapy by administering IL-2 + LAK was more efficient (Rosenberg et al., 1987; Rosenberg, 1988), particularly in terms of the rate of occurrence and possibly also duration of complete regressions. On the other hand, it is known that the administration of IL-2 activates precursors of LAK cells directly in the organism of an individual treated with IL-2 and that, after a several-day cycle of IL-2 infusions, mature, highly efficient LAK cells can be shown in the circulation as well as in organs of the treated individuals. Thus, we can ask what is the advantage of the laborious process where, first, patient's leukocytes are separated by the leukapheresis, then these cells are activated in the laboratory by cultivation in a medium with IL-2 and, last, they are returned into the patient's blood circulation. We can still present no satisfactory answer to this question. One explanation may be that the *in vitro* activation of LAK precursors is more efficient and yields higher numbers of LAK cells with higher cytolytic activity. The other possibility is given by a different activation of different T and NK cell subpopulations under *in vivo* and *in vitro* conditions. From this point of view even much higher IL-2 levels, which can be used for the *in vitro* activation, can play an important role.

Another key problem of the therapy with IL-2 as well as with IL-2 + LAK cells is how to maintain in the organism an efficient therapeutical level of IL-2 for five or more days in spite of the efficient IL-2 inhibitors and feedback mechanisms. The IL-2 level is

Table 6. Systemic IL-2 and IL-2 plus LAK immunotherapy in cancer patients: selected examples from the early experiments

Group	Tumour	No. of patients	Response (%) complete/partial
(A) IL-2			
Rosenberg et al. (1987)	renal carcinoma	21	5/0
	melanoma	16	0/38
Rosenberg (1988)	renal carcinoma	38	11/8
	melanoma	23	0/26
Sosman et al. (1988)	renal carcinoma	17	0/18
Lindermann et al. (1989)	renal carcinoma	11	0/0
	melanoma	18	0/17
(B) IL-2 + LAK			
Rosenberg et al. (1985b)	melanoma	7	14/43
	colon carcinoma	10	0/30
	cervical carcinoma	3	0/100
Rosenberg et al. (1987)	melanoma	26	8/19
	colon carcinoma	26	4/11
	renal carcinoma	36	11/42
Rosenberg (1988)	melanoma	34	9/9
	colon carcinoma	27	4/7
	renal carcinoma	54	13/18
	non-Hodgkin lymphoma	4	25/50
West et al. (1987)	various tumours	40	0/38

necessary for the survival of infused LAK cells and for saving their function (both being incompatible with a longer-lasting decrease of the IL-2 level below a critical value) as well as for the activation and multiplication of other effector cells mediating in the organism the therapeutic effect of IL-2 administration. The NCI group first tried to solve this problem by repeated administration of high doses of IL-2 in the course of the whole therapeutic cycle (Rosenberg et al., 1985b, 1987; Rosenberg, 1988). Another group (West et al., 1987) proposed and successfully tested an alternative solution. They administered lower doses of IL-2 in 24-hour infusions for five days and after a two-day pause the leukapheresis was started. After completing a 3-day activation of LAK cells *in vitro*, they continued giving infusions of IL-2 together with autologous LAK cells for another 3 to 4 days. Although this modification of the IL-2 administration reduced the rate of undesirable side effects after the IL-2 administration and made it possible to apply IL-2 at a common hospital department, not any more at a department of intense care as required by the original (Rosenberg et al., 1987) scheme, the toxicity of the IL-2 administration was not completely avoided; this toxicity is currently a major hindrance to performing the therapy with IL-2 on a lager scale. The pioneer experiments listed in Table 6 were extended and repeated by many groups (for example, Motzer et al., 2000; Chang and Rosenberg, 2001; Gitlitz et al., 2001; Pizza et al., 2001; Lissoni et al., 2002; for a review see Margolin, 2000; Overwijk et al., 2000; Schwartzentruber, 2001; Atkins, 2002), but the results and problems remained, in principle, the same. Only a minority of the IL-2-treated patients responded to the IL-2 therapy; the IL-2 doses required for efficient therapy were very high, close to the toxic doses and produced a variety of adverse effects; finally, the necessary prediction tests that could indicate prior to the IL-2 therapy which patients will respond to the therapy are not available. A partial solution to these problems was expected from the combinations of various cytotoxic and cytostatic agents with IL-2 and from combinations of IL-2 with other synergistic cytokines, which could help to decrease the required high IL-2 doses. Atzpodien et al. (2002a,b) treated 443 metastatic renal cell carcinoma patients with s.c. interferon α (IFNα), low-dose s.c. IL-2, i.v. 5-fluorouracil and oral 13 cis-retinoic acid. The results indicated a long-term (13 years) therapeutic efficacy of the combined therapy in a subset of patients with advanced renal cell carcinoma; however, no significant differences in median overall survival were observed in malignant melanoma patients treated with IL-2 and IFNα versus IL-2 and IFNα plus chemotherapy (Atzpodien et al., 2002a,b). It has been found that renal cell carcinoma patients with progressive metastatic disease after nephrectomy who responded to the IFNα therapy differ from non-responders with regard to *in vitro* sensitivity of peripheral blood lymphocytes (PBL) to IL-2-induced proliferative responses, *in vitro* cytolytic activity of PBL against renal carcinoma cells, and IFNγ secretion by PBL. Whereas the reactivity of the responders was similar to that of control patients without neoplasms, the reactivity of the non-responders was deficient in the examined PBL functions (Indrová et al., 1995; Sobota et al., 1997). These observations may facilitate the identification of renal cell carcinoma patients who are not likely to respond to the cytokine treatment and have an unfavourable prognosis. There is no general consensus with regard to the value of combinations using IL-2 and chemotherapy. Whereas some groups claim positive effects (Gez et al., 2002; de Gast et al., 2003), other groups found the effects of the combination therapy disappointing (Négrier et al., 2000). Another approach utilized to decrease the IL-2 concentration in the circulating blood and, therefore, also the toxicity of the therapeutic IL-2 doses in clinical trials was IL-2 gene therapy.

IL-2 gene therapy

Since vaccines with inserted immunomodulatory genes had not, until a few years ago, been used for treatment of human cancer patients, safety studies had to be performed prior to the vaccination (Anderson, 1992; Thompson, 1993). Schmidt-Wolf et al. (1999) performed the first trial using autologous, IL-2-modified cytokine-induced lymphoid killer cells for treatment of 10 patients with metastatic renal cancer, colorectal cancer and with lymphoma. The phase I clinical study demonstrated that the genetically modified effector cells can be administered without major side effects and that one patient with lymphoma developed a complete response, three patients showed no change by treatment and six patients remained in progressive disease. In another study, thirty-three metastatic melanoma patients were vaccinated according to a phase I-II protocol with an allogeneic melanoma cell line encoding the inserted IL-2 gene. In three patients, vaccination induced inflamatory responses in distant metastases, T-cell infiltration and necrosis/apoptosis. Two other patients experienced complete or partial regression of s.c. metastases. Seven patients had protracted stabilization of metastases. Immune responses could be detected in 67% of the vaccinated patients (Osanto et al., 2000). In the next clinical trial, an allogeneic irradiated renal carcinoma cell line (MRCC) engineered to produce IL-2 was admixed with autologous formalin-treated metastatic tumour cells and used for vaccination of ten MRCC patients. No adverse side effects, one complete and one partial tumour response, as well as two cases of stable disease were observed (Pizza et al., 2003). Recent reviews of worldwide clinical trials for cancer gene therapy (Roth and Cristiano, 1997; Nanni et al., 1999) reported that althogether 27 trials involving IL-2 gene therapy were performed; 49% of all clinical

trials involving cytokine gene transfer used the cloned IL-2 gene (Nanni et al., 1999). However, it is still too early to draw any definitive conclusions from the results of these trials.

Prospects and limitations

The results exemplified in Tables 5 and 6 suggest that the biotherapy with IL-2 is a promising approach to the treatment of selected malignant tumour types, particularly renal cell carcinoma and malignant melanoma. In renal cell carcinoma, combinations of IL-2 and IFNα have shown objective responses in 10–25% of patients (Rosenberg et al., 1989; Atzpodien et al., 1993; Buter et al., 1993; Fyfe et al., 1995; Yang and Rosenberg, 1997; Négrier et al., 1998) and long-lasting responders (Fyfe et al., 1996). Further clinical trials reported an increased response rate when 5-fluorouracil was added to IL-2 and IFNα (Atzpodien et al., 1993; Hofmockel et al., 1996; Joffe et al., 1996; Ellerhorst et al., 1997). The response rates ranged from 16 to 48%. Similar results were reported with metastatic melanoma. Immunotherapy trials with IL-2 had a reported response rate of 15–25%, with a small percentage of durable responses (West et al., 1987; Dutcher et al., 1989; Bar et al., 1990, 1991; Parkinson et al., 1990; Rosenberg et al., 1994a,b). Combination of IL-2 with IFNα or with chemotherapy yielded response rates ranging from 10 to 41%. Response rates exeeding 50% have been reported with chemoimmunotherapy, if the treatment regimens included at least three agents, IL-2, IFNα and cisplatin (for a review, see Keilholz et al., 2000). Inhalatory administration of IL-2 accompanied by s.c. IFNα therapy in renal cell carcinoma outpatients after nephrectomy, as discussed in section 3, was capable of inducing a highly significant percentage of complete and partial regressions of lung metastases (for discussion, see Huland et al., 1997; Bubeník et al., 2000). Experimental studies suggested that primarily non-generalized early forms of cancer, small and very small localized tumours, but also minimal residual tumour disease, micrometastases after resection of the primary tumour, and some forms of haemoblastoses after cytoreductive therapy might be considered for vaccination (for discussion, see Vlk et al., 1998; Bubeník et al., 1999, 2000, 2003; Mikyšková et al., 2004). It should be kept in mind that the conventional modalities of cancer treatment, surgery, chemotherapy, and radiotherapy, are usually accompanied with an early period of transient immunosuppression that may interfere with the immunotherapy. An important conclusion that one can draw from the experimental studies is that the MHC class I-deficient tumours, which represent 40-90% of human neoplasms (Bubeník, 2003) and are resistant to the major effector mechanisms of the tumour defence, $CD8^+$ CTL, can successfully be cured by IL-2 administration (Bubeník et al., 1999, 2003; Mikyšková et al., 2001, 2004; Indrová et al., 2003).

The toxicity of the high IL-2 doses required for the therapeutic effect is still a major obstacle in the therapy of human tumours on a large scale. A possible solution to this problem could be IL-2 therapy with genetically modified, IL-2-producing tumour vaccines. Preclinical studies reviewed in this article have demonstrated that the IL-2 gene may be inserted into the genome of tumour cells, that the genetically engineered tumour cells may secrete therapeutic levels of IL-2, and that vaccination with the genetically modified, IL-2-secreting tumour cells can substantially inhibit tumour growth in a variety of preclinical models and induce immunological memory. The vaccines can be expected to be efficient in a proportion of patients with those types of cancer that respond to the recombinant cytokine therapy. Preclinical studies suggested that safety of viral vectors to be used for the gene delivery should be tested carefully prior to inoculation into humans and that the IL-2-secreting vaccines should be γ-irradiated. Some findings in experimental model systems indicated that IL-2 could, in addition to the activation and proliferation of CTL and T-helper cells required for tumour defence, activate the $CD4^+$ $CD25^+$ T cells and possibly also other supressor mechanisms. The activation and proliferation of the supressor cells could negatively affect the final result of the tumour therapy. Therefore, elaboration of the therapeutic protocols which could prevent the undesirable interference of the tumour inhibitory and immunosupressive mechanisms should also be considered.

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