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Intratumoral low-dose interleukin-2 induces rejection of distant solid tumour

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Summary. This study shows that local tumour treatment with low-dose recombinant interleukin-2 (IL-2) can mediate rejection of a large distant solid tumour. When SL2 lymphoma cells were injected intraperitoneally (i.p.) in syngeneic DBA/2 mice on day 0, 70% of these mice were cured by daily i.p. injections with 20000 units IL-2 on days 10–14. After injecting mice with SL2 both i.p. and subcutaneously (s.c.) on the flank, 50% of the mice treated i.p. with low-dose IL-2 rejected both the i.p. tumour and the large distant s.c. tumour. In contrast, i.p. IL-2 treatment on days 10–14 cured fewer than 10% of the mice bearing only a s.c. SL2 tumour. The described IL-2 immunotherapy also caused systemic tumour rejection in mice bearing both ascitic and solid P815 mastocytoma. Thus it was shown that low-dose IL-2 can induce systemic tumour rejection, when injected at a site of tumour growth. Interleukin-2-induced rejection of s.c. SL2 tumour was highly specific, as mice that were rejecting i.p. and solid s.c. SL2 lymphoma did not reject solid P815 mastocytoma, which was injected s.c. simultaneously on the other flank. Furthermore, solid s.c. tumours consisting of mixtures of SL2 and P815 were not rejected in mice that rejected i.p. SL2 or P815. We conclude that intratumoral injections of low-dose IL-2 can enhance an ongoing weak immune reaction against the tumour resulting in systemic tumour rejection.

Key words: Immunotherapy – Interleukin-2 – SL2 lymphoma

Introduction

Interleukin-2 (IL-2) immunotherapy of cancer has proven to be effective in a number of experimental animal models and clinical trials [2, 13, 17, 23]. In most cases systemically applied high dosages of IL-2 were used; however, the administration of such high dosages of IL-2 leads to severe side-effects [5, 14, 17]. These high doses are necessary to obtain IL-2 levels than can stimulate effector cells at the site of tumour growth. The generation of lymphokine-activated killer (LAK) activity especially demands high IL-2 levels [16, 21], and this LAK activity is thought to be essential for successful immunotherapy with IL-2 in some models [3, 10]. An alternative approach is to administer lower doses of IL-2 at the site of tumour growth. The therapeutic effects of low-dose IL-2 are probably primarily mediated by T lymphocytes [7, 19]. We have shown that immunotherapy with low-dose IL-2, injected at the site of major tumour growth, can cure mice bearing a large burden of SL2 lymphoma [8]. The therapeutic effect of this low-dose IL-2 therapy is most probably not mediated by LAK cells as SL2 cells are LAK-resistant in vitro. In this report we demonstrate that low-dose IL-2, administered at a site of tumour growth, can induce systemic and immunologically specific tumour rejection.

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Materials and methods

Animals. Inbred DBA/2 mice were obtained from Iffa Credo, France. Male mice were used at the age of 6–10 weeks.

Tumours. The DBA/2-derived SL2 lymphoma (of T cell origin) and P815 mastocytoma were used. Both tumours are weakly immunogenic. The in vitro proliferation of SL2 cells is not stimulated by IL-2 [8] and SL2 cells do not produce IL-2 (Maas, unpublished observation). Both tumours grow i.p. as ascitic tumour and were maintained by weekly i.p. passage. From a large number of frozen vials, every month new tumour cells were thawed. These tumour cells were transplanted twice before use in experiments.

Interleukin-2. The recombinant IL-2 (rIL-2) was a gift from Sanofi, Toulouse, France (batch no. IL063P; specific activity 19.8×10^6 units/mg). This IL-2 was produced by Chinese hamster ovary cells, in which the human IL-2 gene was inserted [6]. The IL-2 was diluted to 4×10^4 units/ml in phosphate-buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA; Sigma, St. Louis, Mo.) and stored at −20° C. It was thawed directly before use. PBS contained per liter: 8.75 g NaCl, 1.53 g Na_2HPO_4 and 0.27 g KH_2PO_4.

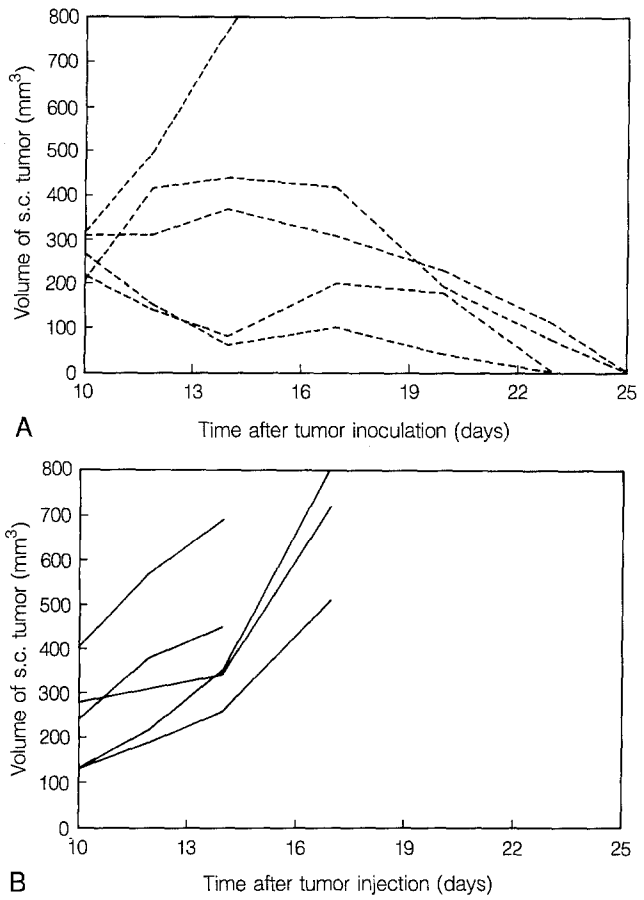

Fig. 1 A, B. Interleukin-2(IL-2)-induced rejection of solid s.c. SL2 tumour. Mice were injected both i.p. (2×10^4) and s.c. on the flank (5×10^5) with SL2 cells. Mice were treated on days 10–14 with daily i.p. injections with 20000 units IL-2 (**A**) or with diluent (**B**). On day 21 all control mice had been killed because of excessive ascites. Five different experiments were performed. Data are shown from a representative experiment

Immunotherapy model. DBA/2 mice were injected i.p. with 2×10^4 SL2 cells or P815 cells on day 0. The tumour cells were suspended in 1 ml RPMI-1640 medium. In some experiments the animals were also injected in the flank at the same time with 5×10^5 SL2 or P815 cells in 0.2 ml s.c. The length (*l*), breadth (*b*) and height (*h*) of the solid tumours was measured every other day. The tumour volume was estimated as $1/6\pi \times l \times b \times h$ (the volume of an ellipsoid body). IL-2 (20000 units/day in 0.5 ml PBS/BSA) was injected i.p. daily on days 10–14 in test animals. Control animals were injected with diluent (PBS/BSA) on days 10–14.

Histology. After dissection tumours were fixed in 4% formalin and embedded in paraffin. Sections of 5 μm were stained with haematoxylin and eosin. In one experiment tumours were fixed in Burckhardt's fixative and embedded in methacrylate. Sections of 3 μm were stained with haematoxylin and eosin.

Results

Low-dose IL-2 induces rejection of distant solid tumour

A previous study had shown that low-dose IL-2 could be very effective in the treatment of mice bearing a large burden of SL2 lymphoma [8]. As SL2 is a metastasizing tumour [4] we concluded that this IL-2 treatment induces systemic tumour rejection. To study IL-2-induced systemic tumour rejection, mice were inoculated with SL2 tumour cells both i.p. (2×10^4 cells) and s.c. (5×10^5 cells). These mice were treated with daily i.p. injections of 20000 units IL-2 on days 10–14. Of the mice treated with 20000 units IL-2/day, 50% rejected both the ascitic and solid s.c. SL2 tumour (Table 1). These mice were able to reject s.c. solid tumours of 200–400 mm³ (Fig. 1). IL-2 injected i.p. cured fewer than 10% of the mice inoculated with s.c. SL2 only (Table 1). This rIL-2 therapy was also highly effective in P815-bearing mice (Table 1). Both ascitic and solid P815 tumour were rejected in 29% of the mice after IL-2 treatment. In addition 36% the P815-bearing mice rejected the ascitic tumour and there was partial response of the s.c. tumour, i.e. a reduction in tumour size of more than 50%. So when low-dose IL-2 is injected at the site of tumour growth it can stimulate an antitumour reaction that can mediate rejection of distant solid tumour.

Table 1. Interleukin-2 (IL-2) immunotherapy of mice bearing ascitic and subcutaneous tumours

IL-2[a] (U)	Tumour[b]		Rejection[c] (%)	
	i.p.	s.c.	i.p.	s.c.
–	SL2	SL2	0/24 (0)	0/24 (0)
20000	SL2	SL2	18/24 (75)	12/24 (50)[d]
20000	–	SL2	–	2/25 (8)[e]
–	P815	P815	0/15 (0)	0/15 (0)
20000	P815	P815	11/14 (79)	4/14 (29)[f]
20000	–	P815	–	0/10 (0)

[a] IL-2 was injected i.p. on days 10–14
[b] 2×10^4 tumour cells were injected i.p. on day 0; 5×10^5 tumour cells were injected s.c. on day 0
[c] Rejection means no visible tumour at day 60
[d] Sum of five experiments in which 2/5, 4/4, 1/5, 1/5, 4/5 mice rejected the s.c. tumour
[e] Sum of five experiments in which 0/5, 0/5, 1/5, 0/5, 1/5 mice rejected the s.c. tumour
[f] 4/14 mice were cured completely in addition 5/14 mice had a partial response i.e. a reduction in tumour size of 50% or more

Specificity of the rejection of solid s.c. SL2 tumour

To investigate the specificity of rejection of the distant solid tumour, mice were inoculated i.p. with 2×10^4 SL2 cells and s.c. with 5×10^5 SL2 cells on one flank and 5×10^5 P815 cells on the other flank on day 0. Treatment with 20000 units IL-2 i.p. on days 10–14 induced rejection of both the i.p. and the s.c. SL2 tumour in 8 out of 10 mice. However, the solid P815 tumour grew progressively in all mice. In Fig. 2 a representative experiment is shown. Thus rejection of the distant s.c. SL2 tumour was highly specific.

Specificity of the tumour cell killing in the solid s.c. tumours

In order to study the specificity of tumour cell killing within the solid tumours, mice were injected s.c. with a

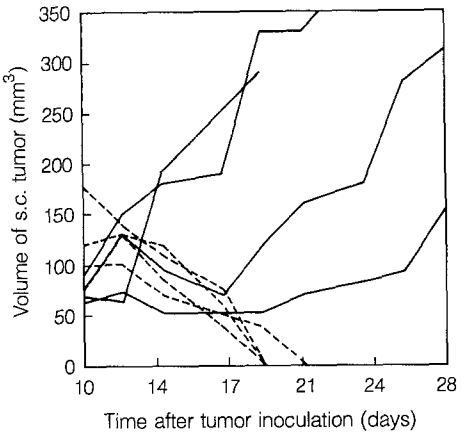

Fig. 2. Specificity of rejection of solid SL2 tumour in mice bearing both solid SL2 (– – –) and P815 (——) tumour. Subcutaneous tumour growth in mice injected i.p. with 2×10^4 SL2 cells, s.c. on one flank with 5×10^5 SL2 cells and s.c. on the other flank with 5×10^5 P815 cells. Mice were treated with daily injections of 20000 units IL-2 i.p. on days 10–14. Two different experiments were performed. Data are shown from a representative experiment

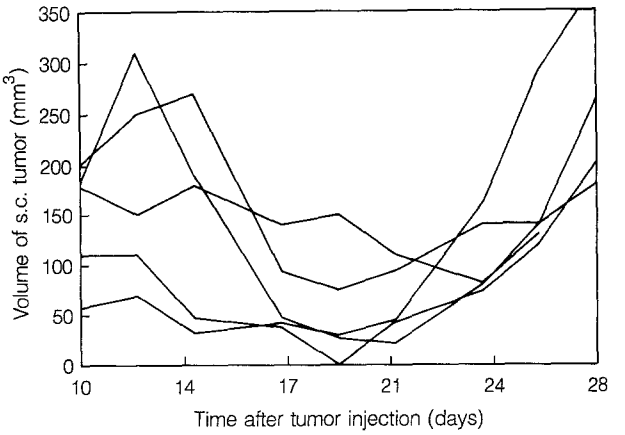

Fig. 3. Effect of i.p. IL-2 therapy on the growth of solid mixed tumours. Subcutaneous tumour growth in mice inoculated i.p. with 2×10^4 SL2 tumour cells and s.c. with a mixture of 2.5×10^5 SL2 cells and 2.5×10^5 P815 cells. All mice were treated with 20000 units IL-2 i.p. daily on days 10–14. Three different experiments were performed. Data are shown from a representative experiment

1:1 mixture of SL2 and P815 tumour cells. When these mice were also injected i.p. on day 0 with SL2, no mice were cured completely. This is in contrast with all five experiments with mice bearing ascitic and s.c. SL2: in all these experiments some mice survived (Table 1). However 9/10 mice bearing i.p. SL2, rejected the ascitic tumour and 8/10 showed a partial regression of the s.c. mixed tumour (Table 2). Some solid, mixed tumours showed a dramatic decrease in volume, but after this initial regression all tumours started to grow again (Fig. 3). Histological examination of the recurring tumours showed that they consisted of P815. When in the peritoneal cavity as well as in the s.c. tumour both SL2 and P815 were present, 2/10 mice were completely cured by IL-2 immunotherapy. Thus it can be concluded that tumour cell killing in the solid tumours is, at least partly, specific.

Histological examination of solid SL2 tumours during IL-2 therapy

To study the histology of the SL2 tumour during rejection, solid s.c. SL2 tumours were dissected from control mice bearing both ascitic and s.c. SL2 tumour and from similar mice treated i.p. with IL-2. Tumours were dissected on days 10, 12, 14, and 17. No differences were seen between control animals and IL-2-treated animals on day 12: the tumour cells were infiltrating the fat and muscle tissue under the skin. However, on day 14 necrosis was seen throughout the solid SL2 tumours of animals treated with IL-2 (Fig. 4). Only a few areas with viable tumour cells were left on day 14. We did not observe tumours with necrosis only at the outer rim of the tumour or only in the center of the tumour. In control tumour-bearing animals very little necrosis was seen. In IL-2-treated mice with regressing s.c. tumours, only small necrotic tumours with very few viable tumour cells were left on day 17.

Table 2. Intraperitoneal IL-2 treatment of mice bearing mixed s.c. tumour

IL-2[a] (U)	Tumour[b]		Rejection of ascitic tumour	Rejection of s.c. tumour[c]	
	i.p.	s.c.		Complete	Partial
–	SL2	SL2/P815	0/10	0/10	0/10
20000	SL2	SL2/P815	9/10	0/10	8/10[d]
–	SL2/P815	SL2/P815	0/10	0/10	0/10
20000	SL2/P815	SL2/P815	5/10	2/10[e]	4/10[f]

[a] Phosphate-buffered saline/bovine serum albumin or 20000 units IL-2 was injected daily on days 10–14

[b] Mice were injected i.p. on day 0 with 2×10^4 SL2 cells or with a mixture of 10^4 SL2 and 10^4 P815 cells. All mice were injected s.c. with a mixture of 2.5×10^5 SL2 and 2.5×10^5 P815 tumour cells

[c] Complete rejection means no tumour is visible on day 60. Partial rejection means at least 50% reduction in tumour volume

[d] Sum of two experiments in which 5/5 and 3/5 mice had a partial response

[e] Sum of two experiments in which 1/5 and 1/5 mice had a complete response

[f] Sum of two experiments in which 2/5 and 2/5 mice had a partial response

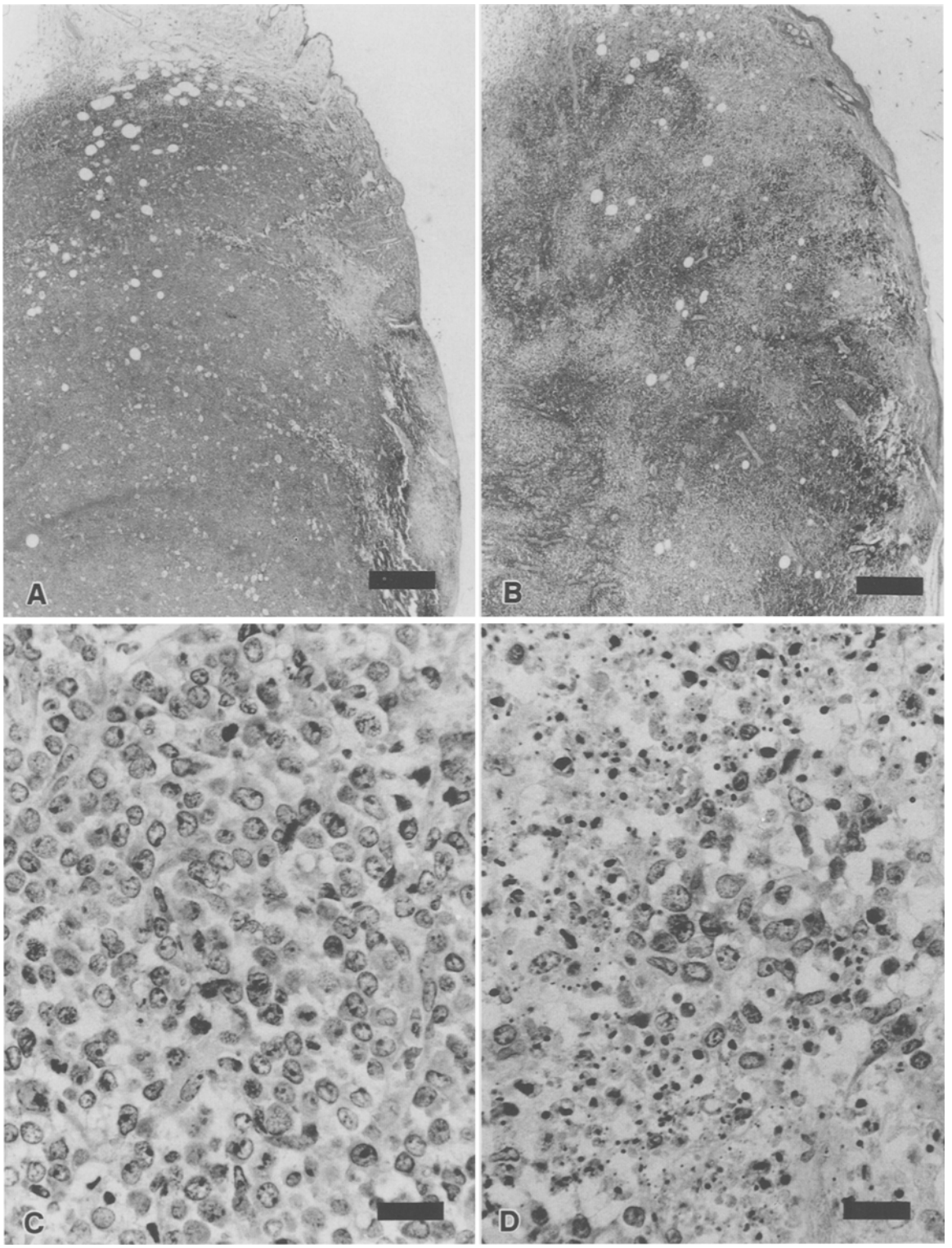

Fig. 4 A – D. Histology of solid s. c. SL2 tumours. The histology of solid SL2 tumours is shown on day 14 after tumour inoculation, from control mice treated with phosphate-buffered saline/bovine serum albumin (**A** and **C**) and from mice treated with daily injections with 20 000 units IL-2 i. p. on days 10 – 14 (**B** and **D**). The *bars* in **A** and **B** represent 450 μm; the *bars* in **C** and **D** represent 45 μm. Tumours in **A** and **B** were embedded in paraffin; tumours in **C** and **D** were embedded in methacrylate

Discussion

In this paper we demonstrate that intratumoral treatment with low-dose IL-2 can effectively stimulate an immune reaction that causes systemic tumour rejection in mice. In SL2-bearing as well as in P815-bearing mice both i.p. ascitic tumours and large s.c. solid tumours were rejected after i.p. therapy with IL-2. The solid SL2 tumours that grew distant from the site where IL-2 was injected were rejected in 50% of the mice when SL2 tumour cells were also present i.p. at the injection site. Furthermore the systemic antitumour effect was highly specific. This means that IL-2 enhances a weak local immune reaction; thereafter, the locally stimulated effector cells of this immune reaction are able to mediate systemic tumour rejection. Interestingly Tanida et al. found an improved therapeutic effect against s.c. growing methylcholanthrene-induced fibrosarcomas when they injected IL-2 i.p. in combination with a cell accumulator attracting effector cells to the injection site [20]. This also illustrates that IL-2 can be very effective in the local stimulation of circulating lymphocytes. In other murine models also, local therapy with low doses of different lymphokines has been reported to enhance a marginal antitumour immune response [1].

In our model low-dose IL-2 can induce tumour rejection because IL-2 is injected at the site where both tumour cells and effector cells are present. In most animal models and clinical trials high-dose IL-2 is given systemically to treat metastatic cancer [9, 17, 21, 23]. The administration of these high doses of IL-2 causes severe side-effects, which are caused primarily by a capillary-leak syndrome induced by IL-2 [5, 14, 17]. This toxicity limits the use of high-dose IL-2. It has also been found in a number of models that low doses of IL-2 can be effective in the treatment of tumour-bearing animals [2, 3, 7, 18]. In most models where low-dose IL-2 is used, only a local antitumour effect is studied. However, some authors have also found a systemic effect. Vaage has reported that s.c. injections of IL-2 can have some systemic effect [22]. However, this systemic effect was transient when non-toxic doses of IL-2 were used. Talmadge has reported that low-dose IL-2 can mediate the regression of both spontaneous metastases and experimentally induced metastases [19]. In this paper we demonstrate that local IL-2 treatment can be highly effective in treating metastatic cancer; it can cause the rejection of large distant tumours.

The specificity of tumour rejection in our model suggests that this IL-2 effect is mediated by T lymphocytes. The specific induction of rejection of the solid SL2 tumours does not necessarily mean that the actual tumour cell killing is solely mediated by specific cytotoxic T lymphocytes (CTL). The described sudden and rapid appearance of necrosis throughout the solid tumours suggests that the initial killing of tumour cells may be mediated by cytotoxic factors rather than by cytotoxic effector cells. Hereafter cytotoxic T lymphocytes may kill the remaining tumour cells. Tumour necrosis factor α (TNFα) seemed a good candidate for the initial tumour cell killing, as the combination of IL-2 and TNFα can be very effective in the treatment of tumour-bearing mice [11, 24]. Since SL2 and P815 cells are not sensitive to TNFα in vitro (unpublished observation) a direct cytotoxic effect of TNFα in our model can be excluded. Still, endogenously produced TNFα might be involved, as TNFα is able to cause necrosis of tumours that are resistant to it in vitro, through effects on the blood vessels in the tumour [12, 15]. However, the exact role of T cells and endogenously produced cytokines in IL-2-induced rejection of solid SL2 tumour remains to be determined.

We conclude that in experimental tumour models immunotherapy with low-dose IL-2 can be highly effective against metastatic cancer, when applied locally at a site of tumour growth. Although we are aware of the differences between experimental and human cancer this finding seems promising for the development of protocols for immunotherapy of human cancer in which low doses of IL-2 are administered and the severe side-effects of treatment with high doses of IL-2 can be avoided.

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