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The Development of Anti-interleukin-2 (IL-2) Antibodies in Cancer Patients Treated With Recombinant IL-2

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Serum samples from 217 cancer patients participating in phase I/II clinical trials were analysed for the development of anti-interleukin-2 (IL-2) antibodies. Patients received recombinant human IL-2 (rIL-2) by continuous intravenous infusion (c.i.v.; $n = 86$) or by subcutaneous (s.c.) injections ($n = 131$). Both patient groups developed anti-rIL-2 antibodies as detected by ELISA with similar frequencies and titres: 52% (median titre, 23) and 47% (median titre, 24), respectively. Using an IL-2-dependent T-cell proliferation assay, sera from 5 c.i.v.-treated patients (6%) and 13 s.c.-treated patients (10%) exhibited neutralising activity. Immunoabsorption studies with rIL-2-coated beads, demonstrated that in 8 of 15 patients with neutralising sera, the neutralising activity was correlated with specific anti-rIL-2 immunoglobulin. All 8 patients had received at least two cycles of rIL-2 by s.c. injections. Specific IL-2 neutralising activity affected both recombinant and natural IL-2 in all 8 patients. Development of anti-rIL-2 antibodies, irrespective of whether these exhibited neutralising activity or not, did not affect the frequency or duration of clinical responses.

Key words: interleukin-2, anti-rIL-2 antibodies, immunotherapy, clinical trials
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INTRODUCTION

ADMINISTRATION OF recombinant cytokines as anti-tumour agents has led to distinct progress in the treatment of selected groups of cancer patients. In particular, treatment of advanced renal cell carcinoma and melanoma patients with recombinant interleukin-2 (rIL-2) has shown promising results with response rates of 16–21% [1–5]. Further improvement of the therapeutic index of rIL-2 therapy is being investigated by adding cytostatic drugs, such as cyclophosphamide [6], 5-fluorouracil (5FU) [7], dacarbazine (DTIC) [8] or other cytokines, such as α and γ interferons (IFN) [9–11], or the administration of lymphokine-activated killer (LAK) cells or *in vitro* expanded tumour-infiltrating lymphocytes [1, 5, 12, 13].

One major obstacle of the therapeutic effect of rIL-2 treatment may stem from the fact that recombinant cytokines often expose potentially antigenic epitopes related to different glycosylation patterns as compared to the native molecules [14]. Recombinant cytokines may, therefore, induce antibody responses which could potentially interfere with their bioavailability and function. Such antibody responses are expected to be particularly frequent with cytokines possessing intrinsic immunological adjuvanticity, such as rIL-2, by providing mitogenic signals to helper T-cells. Indeed, development of anti-rIL-2 antibodies has been reported in 35–90% of patients treated with rIL-2 [15–17].

The present investigation's aim was to determine the frequency with which anti-rIL-2 antibodies developed in a large group of patients treated with this cytokine as part of different phase I/II protocols. Since the administration of rIL-2 by subcutaneous (s.c.) injections on an outpatient basis is introduced as an alternative to continuous intravenous infusion (c.i.v.), we compared the development of anti-rIL-2 antibodies in both c.i.v.- and s.c.-treated patient groups. Cytokine-binding antibodies, as detected by ELISA, may not necessarily interfere with function. We, therefore, used bioassays to determine whether neutralising activity could be detected in ELISA-positive sera. Sera with neutralising activity were absorbed with rIL-2-coated beads to verify whether the observed neutralising activities could be attributed to specific anti-interleukin-2 (IL-2) antibodies. Since the release of soluble IL-2 receptors (sCD25) might also interfere with rIL-2 function [18, 19], we also assessed serum sCD25 levels in selected patients.

MATERIALS AND METHODS

Patients

As part of multicentre studies co-ordinated by EuroCetus (Amsterdam, The Netherlands), patients with progressive metastatic cancer were treated with rIL-2 (EuroCetus) by c.i.v. (86 patients) or by s.c. administration (131 patients). Details on the different protocols used have been previously described and involved c.i.v. rIL-2 alone [1] or combined with LAK cells [1],

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DTIC [8], 5FU [7], and s.c. rIL-2 alone [20] or with IFNα [10] or a tumour vaccine (unpublished). Serum samples were obtained before the onset of therapy, and after the respective courses of rIL-2 administration, but before the next rIL-2 cycle was started. All samples were included which were taken at least 15 days after the first rIL-2 infusion (c.i.v.) or at least 21 days after the first rIL-2 injection (s.c.). Duration of the courses varied from 2 weeks followed by 3 weeks rest for c.i.v.-treated patients, to 6 weeks followed by 2 weeks rest for s.c.-treated patients. Cumulative doses of rIL-2 per cycle were between 90 and 180 $\times$ 10^6 U/m^2 rIL-2 (c.i.v. treatments), or between 144 $\times$ 10^6 and 173 $\times$ 10^6 U/m^2 rIL-2 (s.c. treatments).

Clinical response assessment

Both anti-tumour response and toxicity assessments were evaluated after each treatment cycle according to the World Health Organisation criteria for results of cancer treatment [21].

ELISA for anti-IL-2 antibodies

Presence of rIL-2-binding antibodies in the sera was studied by ELISA. Flatbottom microtitre plates (Nunc, Roskilde, Denmark) were coated with rIL-2 [100 μl/well, 18 $\times$ 10^4 U rIL-2 (specific activity 18 $\times$ 10^6 U/mg) /ml 50 μM Na_2CO_3, pH 9.6] for 18 h at 2–8°C. Plates were pre-incubated for 45 min at 37°C with phosphate buffered saline (PBS)/ bovine serum albumin (BSA) 4% (100 μl/well). One hundred microlitres of serial dilutions (in PBS/BSA 1%) of control and patient sera were added and the plates incubated for 45 min at 37°C. Rabbit-α-human(Ig)-horse radish peroxidase (RαHu-HRP; Dako, Glostrup, Denmark) was bound to the complex for 45 min at 37°C, and the plates developed with *o*-phenylene diamine (OPD). Colour reactions were stopped by addition of 1N H_2SO_4 after 5–15 min, and measured at 492 nm with a Titertek Multiscan (Flow Laboratories, Herts, U.K.).

All sera were routinely screened using duplicate 16-fold dilutions, after which those sera with absorbances greater than 0.5 were retested using 3-fold serial dilutions. The ELISA titre was defined as the product of absorbance and dilution factor, when the absorbance was between 0.1 and 0.5. ELISA titres exceeding 10 were considered positive. Sera of healthy control donors showed ELISA titres between 1 and 4 (n = 10), whereas a positive control serum, obtained by pooling sera from four positive donors, had an ELISA titre between 60 and 70. For the assessment of the incidence of ELISA positivity, any positive ELISA per patient prevailed over all negative samples.

To determine the subclass of anti-rIL-2 antibodies, 100 μl of serial dilutions (in PSB/BSA 1%) of control and patient sera were added to rIL-2-coated microtitre plates, and the plates were incubated for 45 min at 37°C. Swine-α-human IgG 1, 2, 3 and 4 (Nordic, Tilburg, The Netherlands), or RαHu-HRP, IgM and IgG (Dako) were bound to the complex for 45 min at 37°C, after which the plates were developed and evaluated as described above.

IL-2 neutralisation assay

IL-2 neutralising activity was assayed in a proliferation assay with HT-2 cells, an IL-2-dependent murine T-cell line. In 96-well microtitre plates (Costar, Cambridge, Massachusetts, U.S.A.), 10 $\times$ 10^3 cells/well were cultured (24 h at 37°C in 5% CO_2) in the presence of rIL-2 (6 U/ml) or natural IL-2 (Lymphocult T, 0.5 U/ml; Biotest, Dreieich, Germany). The culture medium consisted of RPMI 1640 (Gibco, Gaithersburg, U.S.A.) supplemented with 2 mM glutamine, 100 U penicillin/ml, 100 μg streptomycin/ml and 10% heat-inactivated (30 min at 56°C) fetal calf serum (FCS; Gibco). Heat-inactivated patients' sera or reconstituted serum fractions were added in triplicate at the start of the cultures at a concentration of 2.5% (% v/v). During the last 4 h of culturing, 0.4 μCi/well [^{3}H]methyl thymidine ([^{3}H]TdR; Amersham International, Amersham, U.K.) was added. The cells were harvested with a semi-automatic tissue culture harvester (Skatron, Tranby, Norway) and counted in a liquid scintillation counter. [^{3}H]TdR incorporation was used as parameter for cell proliferation.

Sera were classified as neutralising when percentage [^{3}H]TdR uptake was decreased to below 50% of [^{3}H]TdR uptake observed in the presence of sera of healthy donors. For testing the neutralising activity of low and high molecular weight (putatively Ig-containing) serum fractions, sera were filtrated using Centricon 30 and Centricon 100 microconcentrators (Amicon, Danvers, Massachusetts, U.S.A.), providing fractions with molecular weights below 30 kD, from 30–100 kD and above 100 kD, respectively.

Absorption of anti-IL-2 antibodies

Recombinant IL-2 (27 $\times$ 10^6 U/ml) in coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3) was added to swollen Sepharose 4B-CNBr gel (Pharmacia, Freiberg, Germany) in 1 mM HCl (rIL-2: gel (v/v) = 1:2). The mixture was rotated end-over-end overnight at 4°C. Excess ligand was washed away with coupling buffer, and remaining active groups were blocked by 0.2 M glycine (pH 8.0), incubating for 2 h at 4°C. For absorption of anti-IL-2 antibodies, anti-IL-2 ELISA-positive sera were added to IL-2-coupled Sepharose gel (serum: gel(v/v) = 1:1) and incubated overnight at 4°C. Anti-IL-2-depleted sera were subsequently tested by αIL-2 ELISA and in the HT-2 proliferation assay.

ELISA for soluble IL-2 receptors

Presence of soluble IL-2 receptors (sCD25) in the sera was studied by an ELISA as modified by Hintzen and colleagues [22], using anti-CD25 MAb TB-30 for coating the plates, and biotinylated MAb 7G7B6 for detection (kindly provided by R. van van Lier, CLB, Amsterdam). Concentrations of sCD25 were expressed as U/ml, by reference to a standard curve that was constructed from absorbance values of serial dilutions of a standard provided with a commercially available sCD25 ELISA kit (T-Cell Science).

RESULTS

Development of rIL-2-specific antibodies in rIL-2-treated patients

Sera from 217 patients treated with rIL-2 were tested for the development of anti-rIL-2 antibodies by ELISA. None of the patients' sera collected before initiation of treatment had a positive (titre $\geq$ 10) ELISA titre. Table 1 shows the results after one and two cycles of therapy for each patient group. Sera were available from most patients after one cycle of treatment (66 and 111 of the c.i.v. and s.c. treated patients, respectively), and from part of these and the remaining patients after two or more cycles of treatment (53 and 45 of the c.i.v. and s.c. treated patients, respectively). From the total number of patients (n = 86) who received c.i.v. rIL-2, 45 (52%) developed a positive ELISA score after one or more cycles of treatment. In the s.c.-treated patient group, a similar frequency was observed: 62 of the total 131 available sera (47%). The level of the titres of anti-rIL-2 antibodies did not differ greatly between the patient groups: median titre (range), 23 (12–101) and 24 (11–80) for, respectively, the

Table 1. Development of anti-recombinant interleukin-2 (rIL-2) antibodies after continuous intravenous infusion (c.i.v.) or subcutaneous (s.c.) rIL-2 treatments

Protocol	% ELISA positives after one cycle	% ELISA positives after ≥ two cycles	Total % ELISA positives	% with neutralising antibodies
c.i.v. rIL-2				
IL-2 alone	58% (15/26)	67% (18/27) *	58% (26/45)	0% (0/45)
IL-2 + LAK	50% (11/22)	57% (8/14)	50% (11/22)	0% (0/22)
IL-2 + chemotherapy	33% (6/18)	33% (4/12)	42% (8/19)	0% (0/19)
c.i.v. total	48% (32/66)	57% (30/53)	52% (45/86)	0% (0/86)
s.c. rIL-2				
IL-2 alone	39% (9/23)	67% (6/9)	42% (10/24)	0% (0/24)
IL-2 + IFN-α	41% (34/82)	64% (21/33)	49% (48/98)	8% (8/98) †
IL-2 + tumour vaccine	50% (3/6)	33% (1/3)	44% (4/9)	0% (0/9)
s.c. total	41% (46/111)	62% (28/45)	47% (62/131)	6% (8/131)

* Not all of these patients could be evaluated after one cycle (see text). † None of the sera collected after one cycle of rIL-2 administration contained neutralising anti-rIL-2 antibodies. LAK, lymphokine-activated killer cells; IFN-α, interferon-α.

c.i.v.- and s.c.-treated patient groups. From Tables 1 and 2, it can be concluded that the development of anti-rIL-2 antibodies was not only independent from the route of administration (c.i.v. or s.c.), but also from the tumour types studied (renal cancer, melanoma and miscellaneous) and from the different protocols used: rIL-2 alone, or combined with LAK cells, chemotherapy, IFN-α or a tumour vaccine. A slight reduction in the frequency of antibody formation could be noted in the subgroup of patients who received c.i.v. rIL-2 with chemotherapy, but this difference did not reach statistical significance.

Neutralising activity in rIL-2 ELISA-positive patient sera

All rIL-2 ELISA-positive sera were tested for neutralising activity in the HT-2 proliferation bioassay: 78 sera collected after the first cycle of treatment (32 and 46 after c.i.v. and s.c. treatments, respectively), and 58 sera collected after two or more cycles of rIL-2 treatment (30 and 28, respectively). Sera of 4 patients (2 in each group), collected after one cycle, and sera of 14 patients (3 and 11 of the c.i.v. and s.c. groups, respectively), collected after two cycles of rIL-2 treatment, inhibited rIL-2-dependent proliferation of HT-2 cells to below 50% of the normal serum control value (Figure 1, before absorption).

In order to verify whether the neutralising activities could be ascribed to anti-rIL-2 immunoglobulins, 15 of the 18 neutralising sera were retested in the HT-2 bioassay after absorption with IL-2-coupled Sepharose beads. After absorption, all sera showed anti-IL-2 ELISA titres below 20% of the initial values (data not shown). Eight of the 15 neutralising sera showed at least a 2-fold decrease of inhibitory activity after absorption, indicating the presence of neutralising anti-rIL-2 antibodies (Figure 1). It should be noted that these eight sera had all been obtained from patients who had received at least two cycles of s.c. rIL-2, one of which in combination with IFN-α (Table 1). Importantly, all these eight sera were similarly active in neutralising natural IL-2-induced proliferation of HT-2 cells, which could also be partially reversed by pre-absorption of the sera with rIL-2-bound Sepharose (data not shown). The observed neutralising activity was not significantly related to the ELISA titre (median 29, range 13–80).

In separate experiments, the high molecular weight nature of

Table 2. Development of anti-recombinant interleukin-2 (rIL-2) antibodies after continuous intravenous infusion (c.i.v.) or subcutaneous (s.c.) rIL-2 treatments of patients with different tumour types

Protocol	Tumour type	% ELISA positives	% with neutralising antibodies
c.i.v. rIL-2			
	Renal	56% (35/62)	0% (0/62)
	Melanoma	38% (5/13)	0% (0/13)
	Other	45% (5/11)	0% (0/11)
	Total	52% (45/86)	0% (0/86)
s.c. rIL-2			
	Renal	47% (44/93)	5% (5/93)
	Melanoma	50% (13/26)	4% (1/26)
	Other	42% (5/12)	17% (2/12)
	Total	47% (62/131)	6% (8/131)

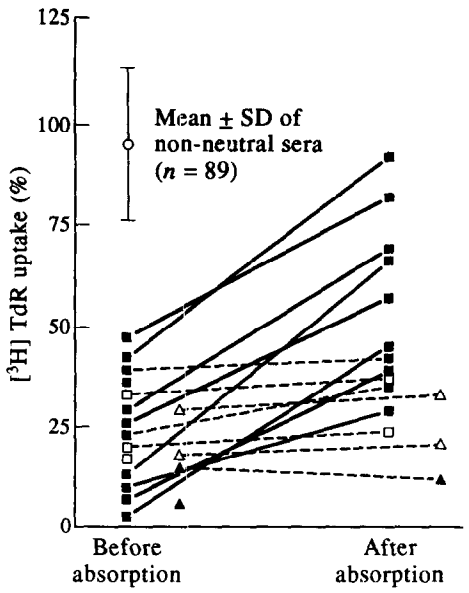

Figure 1. Percentage inhibition of recombinant interleukin (rIL)-2-induced proliferation of HT-2 cells in the presence of non-neutralising sera (n = 89, mean ± S.D. 95 ± 20: ○) and sera from 18 patients scored positive for neutralising activity (≥ 50% inhibition: before absorption). Fifteen of these 18 sera were also tested after absorption to rIL-2-coated Sepharose beads (after absorption). Sera had been obtained from c.i.v.-treated patients, collected after 1 (△) or ≥ 2 (□) cycles, and from s.c.-treated patients, collected after 1(▲) or ≥ 2 (■) cycles. Each serum which showed at least a 2-fold reduction in neutralising activity after absorption is indicated by a solid line. Sera from which no neutralising activities could be absorbed are indicated by a broken line.

the anti-IL-2 antibodies was verified with filter-fractionated serum samples. As expected, in the sera of the 8 patients judged to contain specific IL-2 neutralising antibodies, the anti-IL-2 activity was localised in the high MW fraction. In contrast, it was found that in the remaining seven sera, containing rIL-2 neutralising factor(s), which could not be absorbed by rIL-2-coated Sepharose beads, the neutralising activity was located both in the high and in the low molecular weight fractions (data not shown).

Since soluble CD25 (sCD25) can also bind to IL-2-coupled Sepharose beads and might contribute to the neutralising activity, we measured the presence of sCD25 in neutralising and non-neutralising serum samples. No significant differences were observed in sCD25 titres between neutralising and non-neutralising sera (1124 ± 260 U/ml, n = 6 and 1316 ± 440 U/ml, n = 9, respectively).

Anti-rIL-2 immunoglobulin (sub)class distribution in neutralising and non-neutralising sera

To investigate whether the neutralising activity found in distinct anti-rIL-2 immunoglobulin containing sera might be associated with the presence or dominance of a particular Ig (sub)class, the eight neutralising sera and 44 randomly selected, non-neutralising ELISA-positive samples were analysed by ELISA for IgM and IgG antibodies. In addition, the eight neutralising sera and 13 of the non-neutralising sera were screened for IgG1, 2, 3 and 4 anti-rIL-2 antibodies. No association could be demonstrated between the titres of distinct anti-rIL-2 Ig (sub)classes and the presence of neutralising activity. IgG antibodies of all subclasses were found in 88–100% of all neutralising sera (titres between 33 and 36) and non-neutralising sera (titres between 28 and 39). IgM antibodies were present at slightly lower levels in 38% (3/8; titres between 18 and 25) and 24% (11/44; titres between 19 and 26) of the respective sera.

Clinical responsiveness in relation to the development of anti-rIL-2 antibodies

After one cycle of rIL-2 treatment, anti-rIL-2 antibodies as measured by ELISA had developed to a similar extent in patients showing different clinical responses. No correlation could be found between the development of anti-rIL-2 antibodies and clinical responsiveness, either in the c.i.v.- or in the s.c.-treated patients groups (see Figure 2a). A similar picture emerged when clinical responses were evaluated after the second cycle of treatment (Figure 2b). Notably, most of these patients were already ELISA-positive at the onset of the second treatment cycle. In addition, progression free survival (PFS) time was not affected by the development of anti-rIL-2 antibodies: for those patients classified as stable disease (SD), partial response (PR) and complete response (CR), median PFS was 6.2 months (range 2.5–34.5+) and 10.2 months (range 3.1–24.7) for, respectively, ELISA-negative and -positive c.i.v.-treated patients, and 5.3 months (range 1.5–19.5+) and 6.5 months (range 2.8–32.0+) for, respectively, ELISA-negative and -positive s.c.-treated patients.

Importantly, the observation that 3 of the 8 patients who had developed neutralising antibodies showed an objective response (CR/PR) could support the view that neutralising anti-rIL-2 antibodies do not necessarily interfere with clinical responsiveness. Also, the PFS time in these 3 patients (7.1, 26+ and 32+

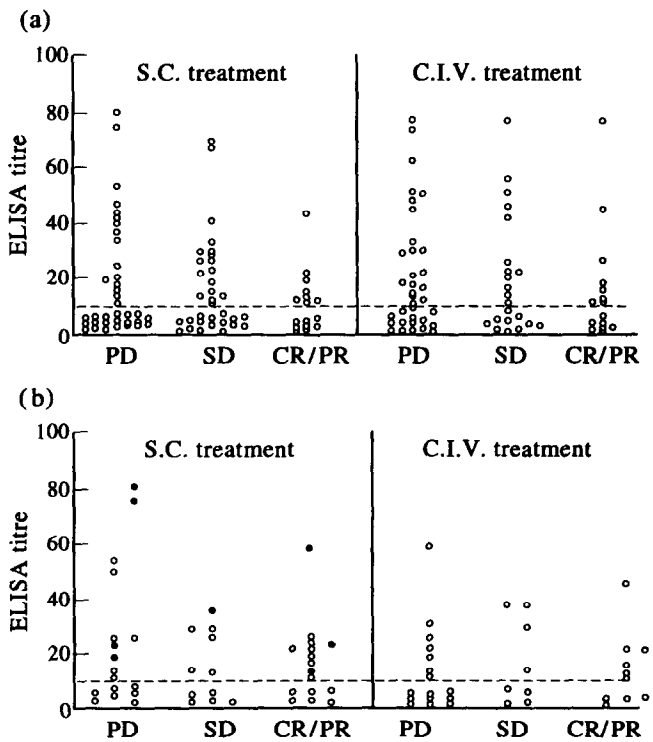

Figure 2. Clinical responsiveness after 1 (a) or 2 (b) cycles of treatment in relation to the development of anti-recombinant interleukin-2 (rIL-2) antibodies. Sera were collected after one or two cycles of rIL-2 administration and tested by anti-rIL-2 ELISA. Eight subcutaneously (s.c.)-treated patients developed neutralising antibodies after two cycles (●). PD, progressive disease; SD, stable disease; CR, complete response; PR, partial response.

months) is comparable to the median PFS time observed in the s.c.-treated patients with objective responses in the absence of neutralising antibodies (8.7 months, range 3.5–21.5).

DISCUSSION

Several conclusions can be drawn from the present study on the development of anti-rIL-2 antibodies in cancer patients treated with rIL-2. Since no significant differences were observed between the different tumour types studied, nor between treatments with rIL-2 alone or with flanking modalities, data for the different c.i.v. and s.c. subgroups of patients have been pooled to substantiate these conclusions.

Firstly, anti-rIL-2 antibodies, as detected by ELISA, were induced with similar frequencies (52 and 47%) and titres (median 23 and 24) in cancer patients treated either by c.i.v. infusions or s.c. injections with rIL-2. This is remarkable, since the i.v. administration of protein antigens is known for its induction of immunological non-responsiveness, primarily reflected by reduced cell-mediated hypersensitivity reactions, but often accompanied by reduced antibody titres [23]. It is assumed that after i.v. administration of antigens insufficient costimulatory signals are available to responding lymphoid effector cells. The addition of rIL-2 to i.v. administered antigens may restore their full immunogenicity by providing a major mitogenic signal [24]. It is conceivable, therefore, that during i.v. administration of rIL-2, its intrinsic mitogenicity contributes to the development of humoral antibodies to epitopes of the recombinant protein itself, thus resulting in similar antibody titres as observed after s.c. administration.

A second point to emerge from the present study is that neutralising anti-rIL-2 antibodies did not develop after a single cycle of rIL-2 treatment, whether by the c.i.v. or s.c. route. However, after two cycles of s.c. rIL-2, neutralising antibodies had developed in 8 of 45 (18%) patients. Notably, no neutralising antibodies were detected in any of the c.i.v.-treated patients at this stage. It might be that during i.v. administration of rIL-2, circulating sIL-2R molecules bind to the rIL-2, thereby preventing the initiation of an immune response towards the biological active site. Thus, as discussed above, the c.i.v. administration of rIL-2 may not induce a lower frequency or level of anti-rIL-2 antibody production, but may nevertheless trigger a different antibody repertoire, not involving IL-2 bioactive sites. Interestingly, the development of neutralising antibodies was not reflected by different profiles of anti-rIL-2 immunoglobulin (sub)classes. Since all patients developing neutralising antibodies had received IFNα in addition to rIL-2 s.c., we cannot exclude the possibility that this addition might have contributed to the development of neutralising antibodies. However, in a recent series of patients (n = 40), we observed that treatment with s.c. rIL-2 alone can also induce neutralising anti-rIL-2 antibodies in 15% of the patients (Scharenberg and colleagues, unpublished results). No sera were available of patients receiving IFNα in addition to rIL-2 by intravenous infusion to challenge the routing hypothesis.

Thirdly, neutralising activity observed in rIL-2-treated cancer patients is not necessarily due to anti-rIL-2 antibodies. Although we excluded a significant contribution of soluble IL-2 receptors released into the serum after treatment with rIL-2, other non-immunoglobulin factors have been described which can interfere with *in vitro* IL-2 bioassays. In the literature, both in tumorous effusions and in sera from cancer patients, neutralising activities have been described not related to anti-IL-2. The molecular weights of these non-specific factors, including p15E and TGFβ, cover a broad range, from below 10 to over 100 kD [25–28]. Indeed, we found seven of 15 ELISA-positive sera to contain neutralising activities with a broad range of molecular weights, which could not be absorbed by an rIL-2 immuno-absorbens, disproving their specific anti-rIL-2 antibody nature. These results show that statements about cytokine-specific, neutralising immunoglobulins should be based on tests which include verification of the antibody nature of the serum factors.

Finally, in the present study, no reduced clinical responsiveness nor PFS time was noted amongst the patients developing anti-IL-2 antibodies as compared to the patients who did not develop these antibodies. In fact, it has been proposed that anti-rIL-2 antibodies could contribute to a positive therapeutic response, by acting as carriers for cytokines and thus retard cytokine elimination from the circulation [29]. Moreover, no indications were obtained for reduced clinical responsiveness in the small patient group (n = 8) developing neutralising anti-IL-2 antibodies, even though natural IL-2-induced proliferation of the HT-2 cell line was inhibited to a similar extent in these patients. No data on pharmacokinetics are available for the patients tested and, therefore, further studies should establish to what extent the emergence of antibodies to IL-2 influences its biodistribution and elimination.

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Survival in Patients With Recurrent Glioma as a Measure of Treatment Efficacy: Prognostic Factors Following Nitrosourea Chemotherapy

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The assessment of efficacy of treatment in patients with recurrent glioma is notoriously difficult, and survival is the most objective endpoint. Between 1970 and 1992, a cohort of 211 patients with recurrent glioma received nitrosourea-based chemotherapy at the time of disease progression. The median survival from the start of chemotherapy was 7 months, with 30% 1-year and 10% 2-year survival probabilities. One-year survival was 22% in 147 patients with recurrent high-grade astrocytoma, 41% in 37 patients with low-grade astrocytoma and 45% in 24 patients with oligodendroglioma. Age, histological grade and Karnofsky performance status (KPS) at recurrence were independent prognostic factors for survival on multivariate analysis. Based on patients' age, tumour grade and KPS, it was possible to define three distinct prognostic groups with 1-year survival probabilities of 60, 21 and 17% ($P < 0.005$). Response to chemotherapy was difficult to assess but correlated with prognostic subgroup, with highest response rate (46%) in the most favourable group and lowest (13%) in the poor prognostic group. In patients with recurrent glioma, patient and tumour parameters are the major determinants of outcome which are identical to prognostic factors at the time of primary diagnosis. They can be used to provide prognostic information for the individual patient, and to stratify patients particularly in trials assessing the efficacy of novel treatments.

Key words: recurrent glioma, chemotherapy, prognostic factors
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INTRODUCTION

THERE ARE many treatment approaches in patients with recurrent glioma. They include either systemic chemotherapy and biological therapy, or local treatment in the form of surgery [1], irradiation (conventional external beam radiotherapy, stereotactic radiotherapy [2] or brachytherapy [3]) and intralesional chemotherapy or biological therapy [4]. The diversity of treatments in patients with recurrent glioma attests to the absence of a regimen with dramatic effectiveness and acceptable toxicity, and patients continue to have poor prognosis.