Alteration of Interleukin 2 (IL-2) Pharmacokinetics and Function by IL-2 Antibodies Induced after Treatment of Colorectal Carcinoma Patients with a Combination of Monoclonal Antibody 17-1A, Granulocyte Macrophage Colony-Stimulating Factor, and IL-2

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ABSTRACT

In this study, we have assessed the development of neutralizing and non-neutralizing interleukin 2 (IL-2) antibodies in metastatic colorectal carcinoma patients receiving a colon carcinoma reactive monoclonal antibody (17-1A) in combination with granulocyte macrophage colony-stimulating factor and IL-2 therapy. Before treatment, no IL-2 antibodies were detected in any of the patients. After therapy, 10 of the 19 patients tested developed antibodies that bound to the IL-2 product used for therapy, but only one developed antibodies that neutralized the biological activity of IL-2 as assessed using an in vitro bioassay. We found that the induction of IL-2 antibodies in some patients irrespective of their neutralizing potential had a significant impact on IL-2 pharmacokinetics. A significant reduction of the area under the concentration-time curve and maximum concentration (Cmax) and increased IL-2 distribution and clearance were observed in IL-2 antibody-positive patients in comparison with IL-2 antibody-negative patients. A significant decrease in IL-2-mediated expansion of lymphocytes was also evident in patients positive for IL-2 antibodies in comparison with those negative for these antibodies. Further characterization of sera from patients with antibodies showed that, in most cases, the antibodies recognized different IL-2 preparations. Results also showed that serum IL-2 concentration at initiation of therapy in patients was significantly higher relative to healthy control donors. The endogenous production of IL-2 gradually increased during the treatment cycles. To conclude, induction of neutralizing and non-neutralizing antibodies in cytokine-treated patients should be carefully monitored in terms of their clinical significance.

INTRODUCTION

In CRC, novel treatment strategies are being explored at present for induction of tumor regression in patients with advanced disease. One immunotherapeutic approach that is being evaluated is combination regimens involving cytokines and MAbs (1, 2). In previous trials, addition of IL-2 or GM-CSF to MAb therapy enhanced the antitumor response in CRC and malignant melanoma patients (3–5). However, the limited success of this strategy has meant that combination therapies involving two or more cytokines (which may act synergistically) and, in some instances, along with drugs or MAbs are now being evaluated in cancer patients (6, 7).

Therapeutic approaches that involve IL-2 in combination with GM-CSF might be promising for patients with cancer because these cytokines activate different cell types. IL-2 activates lymphocytes, whereas GM-CSF activates granulocytes and monocytes/macrophages and enhances antigen presentation (8, 9). It is possible that activation of multiple effector cell populations may be required for effective tumor destruction and increase in rate of survival. Indeed, in a Phase I trial of CRC patients, the benefits of combining GM-CSF and IL-2 for treatment on clinical outcome has been demonstrated (10). On the basis of these results (10) and those obtained from our previous experience with GM-CSF/MAb combination therapy (5), a treatment protocol that combined GM-CSF, IL-2, and MAb17-1A (specificity for the CO17-1A/GA73-3/Ep-cam antigen) for therapy of patients with advanced CRC was designed (11).

One of the major concerns of cytokine-based therapies is...
the induction of antibodies to the therapeutic cytokine(s), which may cause adverse effects in recipient(s). It has been shown previously (12) that treatment with GM-CSF can induce formation of GM-CSF antibodies. In nonimmunosuppressed cancer patients on GM-CSF therapy, development of antibodies against GM-CSF impaired the pharmacokinetics of GM-CSF and, in some instances, neutralized the biological effect of GM-CSF and compromised progressive therapy (13, 14). The development of neutralizing antibodies to GM-CSF impaired the ability of GM-CSF to enhance the number of leukocytes, whereas non-neutralizing antibodies had no effect (13–15). It is possible that treatment with IL-2 may also induce antibodies against IL-2 as shown previously (16–19). However, there is little evidence at present to suggest that induction of IL-2 antibodies in patients receiving IL-2 treatment has any influence on pharmacokinetics and pharmacodynamics of IL-2 and affects IL-2 functional responses and therapy (17). Therefore, the clinical importance of IL-2 antibodies needs to be carefully evaluated.

In this study, an evaluation of immunogenicity of IL-2 is particularly important because addition of GM-CSF to therapy with IL-2 (patients given combination therapy of GM-CSF/IL-2/MAb17-1A) may possibly enhance the incidence of antibody formation against IL-2 by augmenting the capacity of antigen-presenting cells to present antigen (9). Therefore, we have investigated the induction of antibodies against IL-2 in GM-CSF/IL-2/MAb17-1A-treated CRC patients and assessed the clinical effect of these antibodies on IL-2 pharmacokinetics and manifestation of IL-2 functional responses.

MATERIALS AND METHODS

Patients. Nineteen patients (11 males and 8 females) with metastatic CRC and a Karnowsky index of ≥80% were included in this Phase IB/II trial. The median age was 59 years (range, 36–73 years). One patient had preoperative irradiation within 2 months from the start of therapy, but all of the others were, except from primary surgery, untreated during the last 2 months before immunotherapy.

Treatment Schedule. rhGM-CSF 250 (µg/m²/day) produced in Escherichia coli (Leucomax; Schering-Plough, Novartis, Kenilworth; specific activity, 1.2 × 10⁸ – 5 × 10⁸ IU/mg protein) was administered s.c. for 10 consecutive days. rhIL-2 (2.4 × 10⁸ IU/m²) produced in E. coli (Proleukin; Chiron, Amsterdam, the Netherlands; specific activity, 1.8 × 10⁷ IU/mg of protein) was administered s.c. twice daily for 10 days. At day 3 of a treatment cycle, 400 mg of MAb 17-1A (mouse IgG₂a; Centocor) was infused i.v. for 30–60 min. The treatment cycle was repeated every fourth week. Four cycles were given. One patient (number 3) received two additional treatment cycles.

Blood Cell Counts. The total numbers of WBCs were counted using a Zeiss standard RA microscope. Two hundred cells were counted. The percentage of WBC subsets was determined by differential count analysis using May-Grunwald and Giemsa staining.

Serum Sampling. Venous blood was collected in sterile tubes. Blood samples for IL-2 pharmacokinetic analyses were collected before the first IL-2 injection and at 2, 3, 3.5, 5, 6, 8, and 10 h afterward. During treatment, samples were taken at days 1 and 10 of every treatment cycle before the first IL-2 injection and after at least 3 h and 6 h (through IL-2 concentration). Serum was separated and stored at −70°C. Serum samples from healthy blood donors were used as controls.

Cytokine Preparations. Recombinant DNA-derived human IL-2 preparations derived from E. coli expression systems were obtained from Chiron Corporation (Emeryville, CA), Biogen S.A. (Geneva, Switzerland), and Amgen (Thousand Oaks, CA). Jurkat IL-2 was obtained from DuPont Corporation (Philadelphia, PA).

Measurement of IL-2. IL-2 levels in sera from patients were assayed in a sandwich ELISA using commercially available antibodies (Genzyme, Cambridge, MA) in a standard format. Briefly, flat-bottomed microtiter plates (96 wells; Costar, Cambridge, MA) were coated with mouse monoclonal anti-human IL-2 antibodies (Genzyme) overnight at 4°C. After blocking with 8% boiled fat-free milk (Semper, Stockholm, Sweden) in PBS (pH 9.7) for 2 h at 37°C, the IL-2 standard (Chiron, Amsterdam, the Netherlands) diluted in human normal serum in phosphate buffer (1:6; pH 7.4) and serum samples were added in duplicates and incubated overnight at 4°C. A polyclonal rabbit anti-IL-2 IgG antibody (Genzyme) in phosphate buffer (pH 7.4; 1:1000) containing 1% fat-free milk was added for 2 h at 37°C. The plates were then reacted for 2 h at 37°C with a goat-antirabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) in phosphate buffer (pH 7.4) with 1% fat-free milk (1:1000). After enzyme reaction at room temperature using p-nitrophenyl phosphate (1 mg/ml; Sigma Chemical Co.) in diethanolamine buffer (pH 9.8), absorbance was read at 405 nm using an automatic ELISA reader (Multiskan PLUS; Labsystems, Helsinki, Finland).

Serum IL-2 concentration of an individual patient at a given day is calculated as the mean value of the serum IL-2 concentration before the first IL-2 injection on that particular day and of that at 3 h and 6 h after the injection (through IL-2 concentration; Ref. 20).

Binding Assay for Detection of IL-2 Antibodies. A solid phase indirect ELISA was used to detect binding anti-IL-2 antibodies. Briefly, flat-bottomed microtiter plates (Costar) were incubated at 4°C overnight with 100 µl/well of IL-2 (Proleukin; Chiron; 0.25 µg/ml) diluted in 50 mM carbonic-bicarbonate buffer (pH 9.7). After three washes with PBS (pH 7.4), PBS containing 1% BSA and 0.05% Tween 20 was added to each well, and the plates were incubated for 1 h at 37°C. Serum samples were diluted 1:20 in PBS/BSA, and 100 µl of the samples were added in duplicates to the wells. The plates were incubated for 2 h at 37°C. After an additional three washes with PBS, 100 µl of alkaline phosphatase-conjugated goat anti-human antibodies (Sigma Chemical Co.) diluted 1:1000 in PBS was added to each well and incubated for 2 h at 37°C. The wells were then washed three times before the enzyme reaction at room temperature using p-nitrophenyl phosphate (1 mg/ml; Sigma Chemical Co.) in diethanolamine buffer (pH 9.8). The absorbance was read at 405 nm in an automatic ELISA reader (Multiskan PLUS; Labsystems).

Immunoblotting of IL-2 Antibodies. SDS polyacrylamide electrophoresis under nonreducing conditions was carried out using 12.5% total acrylamide gels (approximately 3 µg of protein was loaded/track; Ref. 21). IL-2 protein was heated at 100°C in sample buffer for 5 min before electrophoresis. The
separated proteins were transferred to nitrocellulose membranes, and the membranes were blocked using a solution of 5% (w/v) milk powder in PBS for 30 min on a rotary shaker. The blots were then incubated with serum samples (approximate dilution, 1:200 in PBS/milk) from patients (before and after therapy) or a polyclonal sheep antibody to human IL-2 (in-house reagent included as a positive control at a dilution of 1:2000 in PBS/milk in all of the experiments) overnight at room temperature on a rotary shaker, washed five times with PBS/milk, and further incubated with horseradish peroxidase-conjugated anti-immunoglobulin (of appropriate species specificity, e.g., antihuman; Sigma Chemical Co.) at a dilution of approximately 1:2000 in PBS/milk solution for 1 h on a rotary shaker. The blots were finally washed five times with PBS/0.05% Tween 20, and the immunoreactive protein bands were visualized using the enhanced chemiluminescence reagents (Amersham, Bucks, United Kingdom).

**CTLL Assay for Detection of Neutralizing IL-2 Antibodies.** The biological activity of IL-2 was determined using a bioassay based on the CTLL-2 murine T-cell line that proliferates in response to IL-2 (22). Briefly, serial dilutions of various IL-2 preparations (see above) and the WHO International Standard for IL-2 (86/504) were prepared in 50-μl volumes in 96-well microtiter plates. Exponentially growing CTLL cells were washed three times, resuspended to a concentration of 10^5/ml in RPMI 1640 containing 10% FCS, and added in 50-μl aliquots to each well. The plates were incubated for 24 h, pulsed for 4 h with [3H]thymidine, and harvested, and the radioactivity was incorporated into DNA, estimated by scintillation counting. For neutralization, a 2-fold dilution series giving a final dilution of 1:20 to 1:2560 of the patient’s serum was preincubated with IL-2 (2 IU/ml) for at least 1 h before the addition of cells. Sera were heat-inactivated at 56°C for 30 min before use in the assay.

**Pharmacokinetic and Statistical Analyses.** The pharmacokinetic parameters were calculated using WINNOLIN version 2.0 computer software (Pharsight Corp., San Diego, CA). Plasma concentration-time data were analyzed by nonlinear iterative least square regression analysis. Curve modeling was performed according to the classical one or two compartment open models. The AUC (ng·h/ml) was estimated using the trapezoidal rule, whereas other pharmacokinetic parameters were derived from standard formulae: $T_{1/2}$ is the elimination half-life (h; in one compartment model); $t_a$ is the distribution half-life (h); $t_b$ is the elimination half-life (h; used in two compartment model); $C_{max}$ is maximum concentration (ng/ml); $T_{max}$ is time for maximum concentration (h); $Vd/F$ is distribution volume (liter); and $Cl/F$ is total body clearance (liter/h), where $F$ is the bioavailability.

Differences in distributions between groups were tested with the Mann-Whitney $U$ test. For comparison of WBC numbers in treatment cycle one with the last treatment cycle, the paired $t$ test was used. Differences in pharmacokinetic parameters in patients who were negative for IL-2 antibodies with those that were positive for IL-2 antibodies were assessed using the Mann-Whitney $U$ test and the Wilcoxon paired test wherever appropriate. The paired $t$ test (Graph Pad Instat version 3.0;
RESULTS

Detection of IL-2 Antibodies. Before therapy, none of the patients tested had IL-2 antibodies that were detectable using ELISAs or immunoblots. During treatment with the IL-2-containing regimen, 10 of the 19 patients tested developed IL-2 antibodies that were detectable with ELISA and further confirmed by immunoblotting (Fig. 1). The titers of the IL-2 antibodies increased with repeated IL-2 treatment cycles with the highest values noted after the last IL-2 treatment cycle (Fig. 2). After termination of treatment, the IL-2 antibody titers gradually decreased and, at 4–6 months after the last IL-2 treatment cycle, reached values that were observed with sera taken before IL-2 therapy.

In further immunoblotting experiments, we evaluated the capacity of antibodies in patient’s sera to bind other human IL-2 preparations, e.g., rDNA-derived IL-2 mutein (with minor modifications in its amino acid sequence as opposed to the natural sequence IL-2) from a different manufacturer and also rDNA-derived “natural” sequence IL-2. We found that serum from patients who developed binding antibodies against the therapeutic IL-2 product (cysteine at position 125 is replaced by serine) also recognized another IL-2 mutein (in which the cysteine at position 125 is replaced by alanine) and also the unmodified “natural” sequence IL-2, all expressed in E. coli. Fig. 1 demonstrates typical results obtained by the immunoblotting of two rDNA-derived IL-2 preparations, the IL-2 product used for therapy, and the “natural” sequence IL-2 preparation with sera from patients after IL-2 treatment.

Neutralization assays showed that only 1 of the 10 patients had antibodies that were capable of neutralizing the biological activity of the therapeutic IL-2 preparation. Furthermore, IL-2 antibodies from this patient neutralized the biological activity of various rDNA-derived IL-2 preparations (described above) and also that of natural IL-2 derived from phytohemagglutinin stimulation of the human T-cell leukemic line, Jurkat (Fig. 3).

Serum IL-2 Concentration. Before therapy, the serum IL-2 concentration of the patients (n = 18) was 15.20 ± 7.92 ng/ml (mean ± SE; range, 0–137.6; Fig. 4A). In healthy individuals (n = 36), the serum IL-2 concentration was found to be 1.91 ± 0.96 ng/ml (range, 0–30.13; P < 0.01; data not shown).
This value for serum IL-2 concentration in healthy donors is similar to that reported by others (23). During therapy, the concentration of serum IL-2 increased gradually in the patients with successive treatment cycles. High levels of serum IL-2 were detected in patients before initiation (the first IL-2 injection) of each treatment cycle (Fig. 4A). After the first IL-2 injection of each treatment cycle, a further increase in serum IL-2 levels was noted (Fig. 4B). Analysis of the data showed that the IL-2 levels observed in serum after the first IL-2 injection on days 1 and 10 of a treatment cycle appeared to be related to the presence or absence of IL-2 antibodies. We found that the presence of IL-2 antibodies irrespective of their ability to neutralize had a significant impact on the serum IL-2 concentrations because lower levels were detected in patients who developed IL-2 antibodies in comparison with those that did not (Fig. 5).

After the first single s.c. administration of IL-2 (2.4 × 10⁶ IU/m²), the peak concentration ranged from 0.5–20.7 ng/ml, noted after 3.11 h (mean Tₘₙₐₓ). The pharmacokinetic data of IL-2 obtained at day 1 of treatment cycle one after the first injection of IL-2 in patients is shown in Table 1. On subsequent therapy, the pharmacokinetic profile was significantly different in patients who developed IL-2 antibodies (regardless of their neutralizing potential) in comparison with patients with no antibodies. Results obtained at day 10 of cycles II, III, and IV showed that AUC and Cₘₐₓ were significantly lower, whereas V₃ and Cl were significantly higher in patients with IL-2 antibodies as compared with patients with no IL-2 antibodies (Table 2; Fig. 6).

### Table 2: Pharmacokinetics of recombinant IL-2 in patients with CRC at day 10 of treatment cycles II/III/IV

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>IL-2 antibody-negative patients (n = 5)</th>
<th>IL-2 antibody-positive patients (n = 3)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₘₐₓ (ng/ml)</td>
<td>64 ± 3.00*</td>
<td>12 ± 1.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tₘₐₓ (h)</td>
<td>3.82 ± 0.29</td>
<td>3.4 ± 0.61</td>
<td>NS*</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>4.06 ± 1.16</td>
<td>3.66 ± 1.25</td>
<td>NS*</td>
</tr>
<tr>
<td>AUC (ng × h/ml)</td>
<td>528 ± 71</td>
<td>93 ± 19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>0.19 ± 0.03</td>
<td>1.08 ± 0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>V₃ / F (l)</td>
<td>1.11 ± 0.18</td>
<td>5.70 ± 1.20</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Mean ± SD.  
*NS, not significant.

#### Effect of IL-2 Antibodies on WBCs.
In all of the patients, the number of total leukocytes, neutrophils, lymphocytes, and monocytes were assessed at the beginning (day 1) and end (day 10) of each treatment cycle (Table 3). Statistical analysis of the data showed a significant reduction in the increment of lymphocytes (P < 0.05) at the last treatment cycle in comparison with the first cycle in patients positive for IL-2 antibodies irrespective of their neutralization status (Fig. 7). In patients who did not develop antibodies to IL-2, a significant decrease in cell number was not observed. For total leukocytes, a significant decrease in cell number at the last treatment cycle relative to the first treatment cycle was noted in patients regardless of their antibody status (P < 0.05) in both IL-2 antibody-positive and antibody-negative patients. For neutrophils and monocytes, however, no difference in numbers was observed among the two categories of patients.

#### DISCUSSION
In this study, we have assessed the immunogenicity profile of IL-2 in patients with metastatic CRC treated with a combination of the colon carcinoma reactive MAb 17-1A, GM-CSF, and IL-2 (11). Results showed that 53% of the patients (10 of 19) developed binding antibodies against IL-2, but only one patient’s sera was capable of neutralizing in vitro the biological activity of IL-2. In this study, the immune system of the patients should be considered as well preserved because the patients had not received chemotherapy within more than 2 months before the start of therapy and, therefore, were capable of mounting an effective immune response. All of the patients who developed IL-2 antibodies also developed GM-CSF antibodies, whereas an additional five patients developed GM-CSF antibodies but not IL-2 antibodies. All of the patients also mounted a mouse antibody response (Table 4).

Treatment-induced antibodies to IL-2 have been reported previously (17) in varying degrees (0–100%) in patients with advanced disease. In some cases, IL-2 was administered to patients who were pretreated with heavy chemotherapy or who were on intensive chemotherapy/radiotherapy regimens that may have limited their ability to mount an effective immune response (17). The incidence of antibody formation in our study seems comparable with previous studies in which approximately 60% of the patients developed binding antibodies and 10% developed neutralizing antibodies after IL-2 administration at comparable doses by the same route and without addition of...
other cytokines (e.g., GM-CSF/IFN-α and others; Refs. 17, 19). Therefore, simultaneous administration of GM-CSF with IL-2 did not appear to influence development of IL-2 antibodies in patients of this study given MAb/GM-CSF/IL-2 combination treatment.

Analysis of pharmacokinetic data showed that the induction of IL-2 antibodies in MAb/GM-CSF/IL-2-treated patients had a profound impact on the pharmacokinetics of IL-2 in the individuals studied. A significant reduction of serum IL-2 concentration and an increased distribution and clearance of IL-2 was noted in patients positive for IL-2 antibodies (10 patients for binding antibodies but only 1 patient with neutralizing antibodies) in comparison with those who did not develop any IL-2 antibodies. Such impairment of the pharmacokinetic profile of IL-2 in patients despite the non-neutralizing nature of most of the antibodies detected may perhaps be explained by formation of complexes or aggregates between IL-2 and antibodies that may be cleared by the reticuloendothelial system.

The induction of IL-2 antibodies in treated patients also had a negative impact on IL-2-mediated expansion of lymphocytes. The relative increase in lymphocyte numbers was significantly reduced in patients positive for IL-2 antibodies during the last treatment cycle in comparison with the first treatment cycle. This decrease in cell numbers was observed only in patients with IL-2 antibodies, irrespective of the ability of the antibodies to neutralize IL-2, and was not evident in patients negative for IL-2 antibodies. There was a significant decrease in leukocyte numbers at the end of treatment in comparison with cycle 1 in patients, regardless of their antibody status, but the numbers of neutrophils and monocytes remained unaffected.

In previous reports (16, 18), the presence of IL-2 antibodies was associated with decreased lymphokine-activated killer cell activity and reduced frequency of CD16⁺, CD56⁺, and CD25⁺ lymphocytes, as well as of serum levels of soluble IL-2R. These changes were not associated with impaired clinical response to IL-2 therapy.

The reason for immunogenicity of IL-2 is not known. In most published trials, the nonglycosylated IL-2 product as used in this study was used (17). This IL-2 is a mutein lacking an alanine residue at the NH₂ terminus and carrying a serine substitution instead of cysteine at position 125. The molecule is amphipathic and has a tendency to form dimers and sticky high molecular weight aggregates that may perhaps contribute to the induction of antibodies (17). The mode of administration is also an important factor in the immunogenicity of proteins. s.c. administration may be more immunogenic than i.v. injection.

### Table 3 Absolute cell numbers × 10⁹/l (mean ± SE) at days 1 and 10 of different cycles of IL-2 treatment in patients

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IL-2 antibody-negative patients</th>
<th>IL-2 antibody-positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 2</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 10</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>5.84 ± 0.48</td>
<td>34.57 ± 3.35</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.56 ± 0.21</td>
<td>5.05 ± 0.75</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.35 ± 0.05</td>
<td>1.27 ± 0.51</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3.56 ± 0.32</td>
<td>20.64 ± 2.41</td>
</tr>
</tbody>
</table>

* n = 9 in this instance.

### Fig. 7 Relative change in lymphocyte numbers over a 10-day period during progressive cycles of IL-2 therapy in patients. The symbols in the figures denote individual patients with and without IL-2 antibodies. * denotes a patient with neutralizing antibodies. A t test comparison of lymphocytes in the two groups of patients at day 10 of cycle 3/4 relative to cycle 1 showed significant differences in lymphocyte numbers in antibody-positive patients (P < 0.05) but not in antibody-negative patients. No significant differences were seen with monocytes and neutrophils (data not shown).
Cytokines, which activate the immune system, may be more prone to inducing anticytokine responses. Thus treatment with G-CSF rarely induces antibodies, but antibodies have been demonstrated after therapy with GM-CSF and α-IFN (15, 25). The characteristics of the antibodies may have considerable significance in terms of the biological effect and also clinical responses. Patients with chronic myelogenous leukemia, chronic lymphocytic leukemia, and carcinoid tumors who developed high titers of α-IFN-neutralizing antibodies after α-IFN therapy certainly showed treatment failure to α-IFN (26–28). In GM-CSF-treated patients, the development of antibodies with neutralizing characteristics was clearly associated with reduced efficacy of GM-CSF in terms of its ability to enhance neutrophil and eosinophil numbers. This was not observed in individuals who developed binding, non-neutralizing antibodies to GM-CSF (14, 15). Therefore, it appears that for GM-CSF, neutralizing antibodies are clinically significant, whereas non-neutralizing antibodies are not. However, this may not be the case for other proteins because in this study, formation of antibodies against IL-2 in some patients irrespective of their neutralizing characteristics was associated with a significant reduction in serum IL-2 concentration. Furthermore, a significant decrease in IL-2-mediated expansion of lymphocytes was observed at the last treatment cycle relative to the first cycle in patients positive for IL-2 antibodies in comparison with those negative for IL-2 antibodies. Therefore, it appears that in some cases, non-neutralizing antibodies may be equally important clinically as neutralizing antibodies. The clinical significance of antibodies, however, can only be ascertained in vivo thus emphasizing the need for investigating immunogenicity and clinical sequelae in clinical trials involving rDNA-derived biological products.

The reason for the increased levels of IL-2 observed in patients at start of therapy is not clear. A possible explanation may be an ongoing inflammatory reaction in the tumor leading to endogenous IL-2 production. Increased serum levels of IL-2 have been reported previously (29–31) in various inflammatory diseases. Moreover, Weidmann et al. (32) reported that the pharmacokinetics of infused IL-2 is consistent with a two-compartment model, suggesting synthesis of a considerable amount of endogenous IL-2. The pharmacokinetics of IL-2 in our study also conformed to a two-compartment model. The gradual increase in endogenous IL-2 production with time has not been demonstrated previously. However, it has been shown earlier (33) that treatment with GM-CSF in combination with MAb 17-1A increases the intratumoral inflammatory reaction, which may stimulate endogenous IL-2 synthesis. GM-CSF can stimulate T lymphocytes either directly or indirectly via activation of macrophages and consequently increase IL-2 production (9, 34). The biological significance of the gradually increasing IL-2 concentration is not clear.

In summary, IL-2 treatment induced IL-2 antibodies, which despite being non-neutralizing in nature in the majority of cases compromised the in vivo biological effect of IL-2. Because the use of therapeutic cytokines appears to be increasing, induction of antibodies against cytokines has to be evaluated carefully both with regard to the therapeutic effect of the administered cytokine as well as for interference with effects of endogenously produced cytokines and disease progression.

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