Clinical and Biological Effects of Intraperitoneal Injections of Recombinant Interferon-γ and Recombinant Interleukin 2 with or without Tumor-infiltrating Lymphocytes in Patients with Ovarian or Peritoneal Carcinoma[1]

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ABSTRACT

To identify strategies that enhance tumor-specific immunity in patients with ovarian carcinoma, 22 patients received four to six doses of i.p. recombinant IFN-γ (rIFN-γ), 200 μg/m^2 on days 1, 3, 5, 8, 10, and 12, and i.p. recombinant interleukin 2 (rIL-2), either 6.0 × 10^5 IU/m^2 (group A) or 1.0 × 10^5 IU/m^2 (group B), on days 9, 10, and 11. Two patients in group A also received T-cell lines expanded from peritoneal tumor-infiltrating lymphocytes (TILs) obtained after i.p. rIFN-γ/rIL-2 administration. Toxicity was manageable and included five nonhematological grade 3 or 4 events in 22 patients (23%). A patient had normalization of CA-125 values and a progression-free interval of 18 months, after receiving i.p. rIFN-γ/rIL-2 without TILs. Another patient who received i.p. rIFN-γ/rIL-2 plus TILs had stabilization of ascites and intra-abdominal tumors and >50% reduction in serum CA-125 values over 6 months. A third patient who received i.p. rIFN-γ/rIL-2 had stabilization of intra-abdominal tumors and ascites accompanied by CA-125 values of 50 to 100 units over 6 months. T-cell lines for adoptive immunotherapy were developed for only 3 of 20 patients who were treated with rIFN-γ/rIL-2. Large numbers of CD3$^-$CD56$^+$ adherent cells were expanded in rIL-2 in the remaining patients, precluding the development of T-cell lines. i.p. rIFN-γ, either alone or followed by rIL-2, increased proportions of human leukocyte antigen (HLA) class I$^+$ and class II$^+$ tumor cells and increased HLA class I staining intensity on peritoneal carcinoma cells. i.p. rIFN-γ plus rIL-2 also enhanced cytotoxic activity against Daudi and K562 cells and against allogeneic ovarian tumor cells. Increased cytotoxic activity was associated with an increase in the proportion of CD56$^+$ cells. IFN-γ and IL-2 transcripts were expressed more frequently after rIFN-γ and rIL-2 treatment. In addition, the proportions of CD45RA$^+$ (naive lymphocytes) were increased, and CD8$^+$DR$^+$ lymphocytes were increased relative to CD8$^+$CD69$^+$ cells, which were decreased. IL-10 concentrations in peritoneal fluids were increased after treatment with rIFN-γ and the higher rIL-2 dosing (group A) but not in those treated with rIFN-γ and the lower rIL-2 dosing (group B). These results demonstrated that patients with ovarian carcinoma can tolerate treatment with rIFN-γ and rIL-2 and that rIFN-γ alone or rIFN-γ combined with rIL-2 enhances the expression of HLA class I and class II antigens on ovarian tumor cells, although immunosuppressive cytokines, such as transforming growth factor-β and IL-10, may persist. Treatment with rIFN-γ/rIL-2 i.p. did not facilitate the production of TIL-derived T-cell lines *ex vivo*.

INTRODUCTION

Most patients who have stage 3 or 4 epithelial ovarian cancer have persistent or recurrent disease after the first line of chemotherapy. Results from a recent randomized trial suggest that i.p. chemotherapy may offer a survival advantage to patients who have a small amount of residual disease after initial surgery (1). In addition, surgically documented complete responses have been reported after i.p. treatment with certain cytokines and other immunotherapies (reviewed in Ref. 2). Interestingly, i.p. rIFN-γ[4] produced complete

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[4] The abbreviations used are: rIFN-γ, recombinant IFN-γ; IL, interleukin; rIL-2; recombinant human IL-2; TNF, tumor necrosis factor; TGF, transforming growth factor; TCR, T-cell receptor; HLA, human leukocyte antigen; EOC, epithelial ovarian carcinoma; mAb, monoclonal antibody; MOD, mean absorbance; MNL, mononuclear leukocyte; LCA, leukocyte antigen.

responses in 23% of patients for whom previous chemotherapy had failed (3).

Our experiments (4–6) and those of others (7) showed that TILs obtained from certain patients with ovarian carcinoma contain activated T cells (4–6, 8). In those studies, TIL-derived T-cell lines were generated from the peritoneal cavity tumors of certain patients, after expansion *ex vivo* in low concentrations of rIL-2 (600 IU/ml). In certain instances, T-cell lines of the $CD8^+TCR\alpha\beta^+$ phenotype were developed, and these T-cell lines exhibited primarily autologous tumor cell cytotoxicity (9). In other experiments certain TIL-derived T-cell lines produced cytokines, including IFN-γ and TNF-α, after stimulation by autologous tumor cells (6, 8). Treatment of ovarian tumor xenografts with i.p. rIFN-γ and rTNF-α also has shown significant therapeutic activity in a murine model (10).

The main goal of active immunotherapy is to develop an effective antitumor immune response *in vivo*. T-cell antitumor immunity requires recognition by the TCR of T cells of tumor peptide(s) plus self-MHC presented on the surface of tumor cells or antigen-presenting cells and stimulation through B7-CD28/CTLA-4. Down-regulation of MHC antigen expression could interfere with the presentation of tumor antigen epitopes to T cells. Expression of HLA class I and/or HLA class II may be reduced or absent on ovarian carcinoma cells *in vivo* (11). Moreover, HLA class I^+ expression on human ovarian carcinoma cells correlates with T-cell infiltration *in vivo* and T-cell expansion *ex vivo* in low concentrations of rIL-2 (11). rIFN-γ may augment the expression of HLA class I and class II on ovarian carcinoma cells, and i.p. injections of rIFN-γ increase the expression of HLA class II on peritoneal cavity carcinoma cells (12, 13). Furthermore, rIFN-γ enhances the generation of tumor-specific cytotoxic T cells, very likely through the induction of HLA class I and class II (14, 15). Propagation *in vivo* of the tumor-specific CTLs generated by rIFN-γ administration would likely require administration of rIL-2 (16, 17). For these reasons we designed a clinical trial to treat patients with ovarian carcinoma with i.p. rIFN-γ followed by i.p. rIL-2. In addition to the specific T-cell-mediated effects of rIFN-γ, rIFN-γ has been reported to enhance proliferative responses of T cells from certain donors *in vitro* (18, 19), to enhance expression of activation markers on human $CD8^+$ T cells *in vitro* [and in particular IL-2 receptors (Tac antigen) and HLA-DR and allospecific CTLs (18)], and to increase activity of nonspecific killer cells (20–23). These results suggest that i.p. administration of rIFN-γ followed by rIL-2 may induce antitumor activity *in vivo* by enhancing the expression of HLA class I and class II antigens on ovarian carcinoma cells and by inducing proliferative and cytotoxic functions of peritoneal effector lymphocytes.

We, therefore, conducted a clinical trial to determine (*a*) the intensity and frequency of clinical toxicity and of clinical responses after treatment with i.p. rIFN-γ and i.p. rIL-2, either alone or in combination with TIL-derived T-cell lines; (*b*) whether i.p. injections of rIFN-γ and i.p. rIL-2 increased HLA class I and class II expression on ovarian tumor cells *in vivo*; (*c*) whether this treatment facilitated the production of $CD8^+CD4^-TCR\alpha\beta^+$ or $CD4^+CD8^-TCR\alpha\beta^+$ TIL-derived T-cell lines with specific activity *in vitro*; and (*d*) the effect of this treatment on the expression of differentiation and activation antigens on peritoneal exudate cells and on cytokine levels in the peritoneal cavity. The selected i.p. rIFN-γ dose reportedly increased HLA class II^+ expression *in vivo* (12, 13), although quantitative measurements of HLA class I and class II on tumor cells were not performed in these studies (12, 13). The selection of i.p. rIL-2 dosing in the trial was based on our previously reported pharmacokinetic study of i.p. rIL-2 (24), in which bolus doses of i.p. rIL-2 (6.0×10^5 IU/m^2) resulted in peritoneal IL-2 concentrations of 20–30 ng/ml after 24 h.

MATERIALS AND METHODS

Eligibility Criteria

Eligibility criteria included a diagnosis of EOC or Müllerian variants, prior treatment with platinum-based chemotherapy, visible or microscopic disease at reevaluation surgery, with a largest single tumor diameter of 5 × 5 cm (patients could be eligible after tumor debulking), adequate hematological and other organ functions defined by the following: granulocyte count, 1500/μl^3; platelets, 100,000/μl^3; bilirubin, 1.5 mg/dl; creatinine, 1.5 mg/dl; or creatinine clearance, 60 ml/min; Karnofsky performance status, >80%. Exclusion criteria included significant heart disease or arrhythmias, HIV and hepatitis B surface antigen positivity, whole abdominal irradiation, intestinal dysfunction or suspected extensive adhesions from prior history or findings at laparoscopy, overt autoimmune disease, and prior treatment with rIL-2 or rIFN-γ.

rIFN-γ

NSC 60062 was obtained from the Cancer Therapy Evaluation Program at the National Cancer Institute. Specific activity was 30×10^6 units/mg of the noncovalent dimeric form of protein. Each 0.5 ml vial contained 100 μg (3×10^6 U) of IFN-γ1b, formulated in 20 mg of mannitol, 0.36 mg of sodium succinate, 0.05 mg of polysorbate and reconstituted. rIFN-γ (Genentech, Inc., South San Francisco, CA) was reconstituted in 20 ml of sterile water at room temperature, and 200 μg/m^2 were diluted in 300 ml dextrose 5%, 0.025 NS for i.p. administration over 30 min.

rIL-2

Lyophilized Proleukin (aldesleukin, specific activity of 18×10^6 IU/mg of protein; Chiron-Cetus, Emeryville, CA) was first dissolved in sterile USP water and 6.0×10^5 IU/m^2 were diluted in a final volume of 250 ml of dextrose 5% in distilled water with 0.1% human serum albumin for i.p. administration over 30 min. With no clinical ascites, approximately 1.5 liters of D5 0.25 normal saline was injected into the peritoneal cavity prior to i.p. administration of the rIL-2.

Production of TIL-derived T-Cell Lines for Adoptive Immunotherapy

TIL-derived T-cell lines were produced using a four-step expansion procedure under serum-free conditions as described previously (4) and with the following modifications: after 1–2 × 10^9 cells were generated, the TILs were transferred with the media into an AM-FP13EO (lot 939B9F) cartridge of a hollow-fiber bioreactor. This cartridge required lower rIL-2 concentrations than the previous BRB-23B1 System (4). TILs were harvested 19–21 days later. TILs expanded in the bioreactor primarily were either $\alpha\beta TCR^+CD3^+CD4^+CD8^-$ or $\alpha\beta TCR^+CD3^+CD4^-CD8^+$.

Table 1 Dose and schedule for i.p. injections of rIFN-γ and rIL-2

	Week 1					Week 2				
	D1	D2	D3	D4	D5	D8	D9	D10	D11	D12
Group A										
rIFN-γ 200 μg/m^2 >30 min	×		×		×	×		(×)[a]		
rIL-2 bolus 6.0 × 10^5 IU/m^2 >30 min							×	×	×	
Group B										
rIFN-γ 200 μg/m^2 >30 min	×		×		×	×		×		×
rIL-2 1.0 × 10^5 IU/m^2 >6 h							×	×	×	
Time points for immunobiological studies	T1				T2	T3				T4

[a] (×), Additional dose of i.p. rIFN-γ added after the first five patients entered. Patients received TILs (D2) during a cycle that consisted of i.p. rIFN-γ (D1 and D4) and i.p. rIL-2 (D2, 3, 4, 5).

Isolation and Expansion in rIL-2 of CD8$^+$ or CD4$^+$ TIL-derived T-Cell Lines

Purified CD4$^+$ or CD8$^+$ T-cell lines were obtained from rIL-2-expanded TILs using appropriate antibody-coated flasks (CELLector devices; Applied Immune Sciences, Menlo Park, CA; Ref. 25). TILs (2 × 10^8) were transferred in complete AIM V medium (includes rIL-2 at 600 IU/ml) to a CELLector-TIL sterile polystyrene chamber, with anti-CD8 mAb covalently bound to the inner surface. CD8$^+$ cells were retained by the chamber (26), and within 2–4 days, they were detached from the anti-CD8 mAb matrix and continued to expand as described for unseparated rIL-2 expanded T-cell lines (4).

i.p. Catheters. A 5F vascular-type catheter was inserted into the peritoneal cavity under fluoroscopic guidance. Radio contrast was injected to determine free flow in the peritoneal cavity (2). Catheters were placed approximately 3 days before the start of treatment. On their removal, catheter tips were routinely submitted for culture and antibiotic sensitivity.

Study Design

To analyze for the effects of i.p. rIL-2 dosing, patients were divided into two groups (Table 1): Group A patients received four or five i.p. injections of rIFN-γ, dosed at 200 μg/m^2 per injection, and three i.p. injections of rIL-2 during the 2nd week, dosed at 6.0 × 10^5 IU/m^2 per injection, over 30 min. Group B patients, in addition to the i.p. rIFN-γ, received rIL-2 at the reduced dose of 5.6 μg/m^2 (1.0 × 10^5 IU/m^2) given over 6 h. In Table 1, T1–T4 indicate the time points used in immunobiological analyses. The i.p. rIL-2 dose and schedule for group B were expected to result in peritoneal IL-2 concentrations of 50 to 100 IU/ml. Similar concentrations of rIL-2 stimulate the production of TIL-derived T-cell lines (4, 5). Concurrent use of steroids was not allowed. Bactrim or Ciprofloxacin (if the subject was allergic to the former) was given while the i.p. catheter was in place. After the first eight patients had been treated, the protocol was amended to permit patients to receive additional courses of IFN-γ/rIL-2 at the same schedule if their TILs could not be grown *in vitro*. Each additional course, which was separated by an interval of 2–3 weeks, was administered at the same schedule.

This study included 22 patients with ovarian carcinoma. All had received tumor reduction surgery and platinum-based chemotherapy, which for 19 patients also included paclitaxel. Eighteen patients (83%) had elevated CA-125 values (range, 46–3020 units; normal, <37 units) before treatment. All of the patients had tumor that was evaluable surgically or radiologically for response. Only two patients in this study had "minimal residual disease," defined as visible tumors equal to 1 cm and an absence of tumor metastases at multiple sites. Nine patients had ascites. Peritoneal fluid (ascites or washings) were positive for malignant cells in 19 (86%) of 22 patients, and aneuploidy was detected in 8 (40%) of 20 peritoneal fluid specimens tested before treatment. Twenty patients were HLA-typed, serologically. Four (20%) of 20 were HLA-A2$^+$, which is less than the expected frequency of 40–50% in this population. Ten (50%) of 20 were HLA-A1, 4 (20%) were HLA-A11, and 4 (20%) were HLA-A26.

Quantitative Immunohistochemical Analysis of HLA Class I and Class II on Peritoneal Carcinoma Cells

Preparation of Cells. Peritoneal exudate cells were examined during treatment to determine whether i.p. rIFN-γ followed by i.p. rIL-2 resulted in increased *in vivo* expression of HLA class I or II on tumor cells. Ascitic fluid or peritoneal fluid washings were centrifuged at 800 × *g* for 10 min, and the mixed cell pellet was resuspended in PBS (Life Technologies, Inc., Grand Island, NY) and purified on a Ficoll-Hypaque density cushion. Cytospin slides were prepared on a Shandon centrifuge (Pittsburgh, PA), fixed in cold acetone, and stored at −20°C.

mAbs. The following mAbs to HLA were used: anti-HLA class I (W6/32), an IgG2a that recognizes a monomorphic determinant of the HLA class I molecule, and anti-HLA class II (anti-HLA-DR), an IgG1 that recognizes a common framework determinant of the HLA-DR antigen (Dako Corp., Carpinteria, CA). Appropriate isotype control mAbs were used.

Immunohistochemical Staining. This was carried out by an established method (11). Cytospin slides were incubated with primary mAbs (optimum concentration, 0.1 μg/ml) for 2.5 h at room temperature. Slides were washed and incubated with biotinylated antimouse IgG antibody (Vector Laboratories, Burlingame, CA), and after further extensive washing with avidin-biotin-peroxidase complex (Vector Laboratories). Peroxidase activity was developed with freshly prepared diaminobenzidine (0.025%), nickel chloride (0.02%), and hydrogen peroxide (0.0015%) mixture in PBS. Methyl green was used for counterstaining. Controls included cells incubated with 0.1 μg/ml isotype matched irrelevant mAbs (IgG1 or IgG2a).

Quantitation of Immunohistochemical Staining. The density of staining for HLA class I and class II antigens was measured using a SAMBA 4000 image analyzer as we have described previously (27). The significant element in the final report was the MOD, which represents the mean labeling concentration in the labeled areas. Nine fields per specimen that contained tumor cell clusters were used for this analysis, via a grid system. All of the specimens were coded, and analysis was performed in a blinded fashion. Proportions of positive tumor cells were determined as described previously (11).

In Vitro Cytotoxicity of Peritoneal Cavity Effector Cells against Allogeneic and Autologous Tumor Cells

Cytotoxicity was determined at 6.25:1, 12.5:1, and 25:1 effector-to-target ratios using a ^{51}Cr release assay as described previously (4). Target cells included $SKOV_3$ (ovarian), Daudi, and K562 tumor cell lines and, if sufficient numbers were available, autologous ovarian tumor cells.

Cell Surface Differentiation and Activation Antigens

Approximately 400 ml of fluid was removed through the i.p. catheter before the first day of treatment, and MNLs were prepared on a Ficoll-Hypaque density cushion. Proportions of MNLs that expressed differentiation and activation antigens were determined by methods described elsewhere (4, 24, 28). Briefly, the leukocyte population was first identified by its forward-and side-scatter characteristics and by the staining of these cells with mAbs that bind to a common leukocyte antigen (LCA^+ cells), followed by staining of those cells with mAbs recognizing CD3, $CD14^+/CD45^+$ (Becton Dickinson, San Jose, CA). Proportions of $CD3^+$ and $CD14^+$ cells were determined in the whole cell population. A "live gate" was placed on the lymphocyte population (typically contained $<5\%$ $CD14^+$ cells) to determine the proportions of $CD3^+$, $TCR\alpha\beta^+$, $TCR\gamma\delta^+$, $CD56^+$, $CD16^+$, $CD4^+CD45RO^+CD45RA^-$, $CD8^+CD45RO^+CD45RA^-$, $CD4^+CD69^+$, $CD8^+CD69^+$, $CD4^+DR^+$, $CD8^+DR^+$, and $CD25^+$ cells. Proportions of naive T lymphocytes ($CD45RA^+$), and memory cells expressing the late activation antigen $CD45RO^+$, including $CD4^+CD45RA^+$ $CD45RO^+$, $CD4^+CD45RA^+CD45RO^-$, $CD4^+CD45RA^-CD45RO^+$,$CD8^+CD45RA^+$ $CD45RO^+$, $CD8^+CD45RA^+CD45RO^-$, and $CD8^+CD45RA^-CD45RO^+$, also were determined.

Cytokine and Neopterin Concentrations in Peritoneal Fluids

The effects of i.p. rIFN-γ and i.p. rIL-2 on the concentrations of IL-10, TGF-β2, and neopterin was determined by standard ELISA as described previously (24). The following ELISA kits were used: IL-10 (Biosource International, Camarillo, CA), TGF-β2 (R&D Systems, Minneapolis, MN), IL-2 (Amersham, Arlington Heights, IL), rIFN-γ (Biosource), and neopterin (American Laboratory Products Company, Windham, NH).

Nitrate Concentrations in Peritoneal Fluid

Nitrate, a stable metabolite of nitric oxide, was measured by incubating 50 μl of peritoneal fluid with 50 μl of nitrate reductase. In the enzyme, working solution comprised 1 ml of HEPES (pH 7.2), 1 ml of 2.4 M ammonium formate, and 100 μl of *Escherichia coli* nitrate reductase (100 mg/ml; from Dr. E. A. Grimm, M. D. Anderson Cancer Center, Houston, TX), at 37°C in 96-well plates for 30 min. To deproteinize, 150 μl of 1.5% zinc sulfate in water was added, and the plate was centrifuged at $2000 \times g$ for 15 min. Fifty μl of supernatant were transferred to another plate, 50 μl of Griess reagent [1% sulfanilamide and 0.1% napthylethylenediamine (Sigma, St. Louis, MO) in 2.5% phosphoric acid] were added, and absorbance was read at 540 nm after a 5-min incubation. The concentration of nitrate was calculated from a standard curve of different sodium nitrate solutions. Background controls of 25 g/l phosphoric acid instead of Greiss reagent consistently had optic densities lower than that of the lowest standard, which was 5 μg (±SE).

Detection of Transcripts for IFN-γ and IL-2 by Reverse Transcription-PCR

Reverse transcription-PCR for the above transcripts was performed on DNA extracts of peritoneal exudate cells as described previously (8).

Biostatistical Analysis

Results were analyzed by repeated measures ANOVA. To correct for the large number of comparisons that were made, a Bonferroni adjustment was applied twice: to adjust for the number of variables and for the number of comparisons that were made between times for a variable. Of those *n* variables that were determined to have a significant time effect, the raw *P* value for a contrast between two time periods was multiplied by the number of contrasts performed for that variable to obtain the adjusted *P* for a comparison between two time periods. The adjusted *P* was set at 0.05 (29).

RESULTS

Clinical Effects of i.p. rIFN-γ and i.p. rIL-2 Alone or in Combination with i.p. TIL. Twenty-two patients received one or more courses (a total of 41) of the i.p. rIFN-γ and i.p. rIL-2 treatment. In addition, two of these patients also received TILs. TILs were not given to a third patient because of progression. A treatment course comprised four injections of rIFN-γ in 4 patients, 5 injections in 7 patients, and 6 injections in 10 patients. rIL-2 at a dose of 6×10^5 IU/m^2 was given to 11 of the first 12 patients (group A), and 10 (group B) received 1.0×10^5 IU rIL-2 as a 6-h infusion. Because of toxicity, one patient from group A received three i.p. injections of rIFN-γ and 50% of the IL-2 dosing in week 2, and a second patient received 50% of the third i.p. rIL-2 dose. One of 10 patients in group B received a 50% reduction in i.p. rIFN-γ during the second course, because of side effects. Twelve patients in group A received a total of 13 courses and, in addition, 2 courses with TILs, and 10 patients in group B received 28 courses.

Clinical Toxicity. Five (22.7%) of 22 patients experienced grade 3 or -4 nonhematological adverse events. These events occurred either at the higher i.p. rIL-2 dose (1 of 12 patients) or at the lower i.p. rIL-2 dose (4 of 10 patients). These toxicities involved 5 (12.2%) of 41 courses administered without TILs. Intraperitoneal injection of TILs in two patients did not produce any grade 3 or 4 toxic events. Clinical toxicities were reversible and manageable by routine medical treatments.

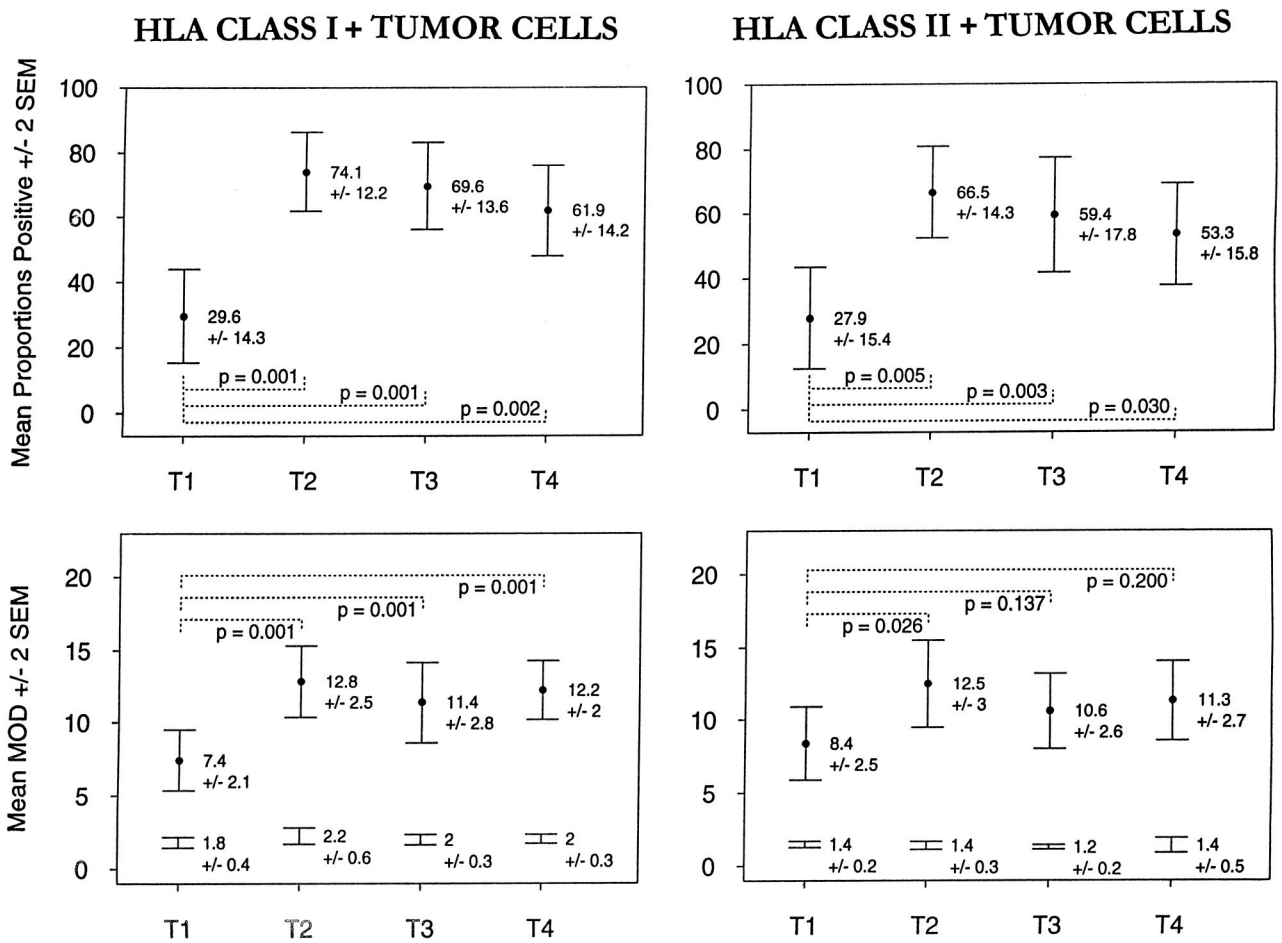

Fig. 1 Effects of rIFN-γ and rIl-2 on the expression of HLA class I and class II antigens on peritoneal carcinoma cells (*top two panels*). *T1*, before treatment: *T2*, 2 days after the second rIFN-γ injection (day 5 on Table 1); *T3*, 3 days after the third rIFN-γ injection (day 10); *T4*, after all of the rIFN-γ and rIL-2 injections (day 12). *Bottom two panels*, MOD values for isotype-matched antibodies to HLA I and II.

There were no treatment-related deaths. One of 12 patients in group A experienced grade 3 neuroconstipation and dehydration associated with anorexia during the first course. Four patients from group B experienced the following significant adverse reactions: reversible cardiac arrhythmia, nausea, malaise, abdominal pain, and hematemesis during a second course (1 patient); significant nausea, anorexia, malaise, and abdominal pain during a third course (1 patient); indigestion (during the first course) and chills (during a second course; 1 patient); malaise and massive ascites (30 liters removed) during a fourth course (1 patient), which resolved after controlled hydration and withdrawal of the i.p. rIFN-γ. This patient continued treatment with rIFN-γ at 50% of the initial dose.

Ten (45%) of the 22 patients experienced 17 catheter-related events over the 43 courses. These events included leakage at the catheter site (4 patients), pain (2 patients), one-way valve effect (3 patients), dislodgment (2 patients), break at the catheter hub (1 patient), and infection (5 patients). Five catheter-related infections were associated with the following organisms: coagulase negative *Staphylococcus* (2 patients), coagulase negative *Staphylococcus* and *Corynebacterium* (1 patient), hemolytic *Streptococcus* and *Xanthomonas maltophilia* (1 patient), and *Klebsiella pneumoniae* (1 patient). All of the patients recovered after administration of appropriate antibiotics and/or removal of the catheter. Six (27%) of 22 patients required catheter replacement for one or more of the following reasons: persistent leakage, pain attributable to catheter position, valve effect, dislodgment, and catheter break.

Hematological adverse events were uncommon. One of 22 patients during the first course and 1 of 13 in the second course experienced grade 3 neutropenia. There were no grade 4 neutropenic or thrombocytopenic events. All of the patients developed lymphopenia to some extent. Grade 3 or 4 lymphopenia occurred in 19 of 22 patients during the first course, in 12 of 13 during a second course, and in 5 of 5 in a third course. Lymphopenia seemed to be associated with an increase in the numbers of MNLs in the peritoneal cavity after treatment with i.p. rIFN-γ and rIL-2. Lymphocyte counts returned to normal values by the next treatment course. The clinical significance of the transient lymphopenia is unknown.

Clinical Responses. One patient from group A who was given TILs in addition to i.p. rIFN-γ and rIL-2 showed stabili-

zation of a pelvic tumor mass and ascites (her peritoneal fluids were positive for malignant cells before treatment but showed only atypical cells after treatment). This patient's CA-125 values dropped from 817 to 348 units during a 6-month period. From group B one patient had ascites regression, and CA-125 values of 3070–3141 units over 3 months; another had normalization of the CA-125 titer and delay in progression of subdiaphragmatic metastases for 18 months found at laparoscopy after six courses of treatment with platinum and paclitaxel. A third patient from group B had stabilization of ascites and abdominal tumor masses and CA-125 values of 50 to 100 units for 6 months. Of eight patients whose peritoneal fluids showed aneuploidy before treatment, four had temporary suppression of the aneuploid component but no reduction in ascites or in CA-125 values.

Increased Proportions of HLA Class I^+ and HLA Class II^+ Tumor Cells and Increased Levels of HLA Expression on Tumor Cells. Tumor cells in peritoneal fluids (ascites or washings) were stained for HLA class I and HLA class II in 12 patients. The numbers of tumor cells in the peritoneal fluid in the remaining patients were insufficient for quantitative analysis. The density of HLA expression (MOD) was measured on peritoneal tumor cells obtained daily from the first five treated patients. From this analysis (data not shown), four time points (Table 1) were identified that seemed to coincide with the peak effect of i.p. rIFN-γ and the cumulative effect of rIL-2: T1, before treatment; T2, 2 days after the second injection of rIFN-γ; T3, 3 days after the third injection of rIFN-γ; and T4, after rIFN-γ and rIL-2. During the study phase when daily measurements of the MOD were made, tumor cells from the first two patients showed a decrease in HLA class I or II expression during the second week after the i.p. injections of rIL-2. Therefore, subsequent patients who were entered later received a fifth dose of i.p. rIFN-γ on day 10. Before the treatment (T1) 29.6 ± 14.3% (mean ± SE), tumor cells were HLA class I^+ and 27.9 ± 15.4% HLA class II^+ (Fig. 1). As shown in Fig. 1, i.p. injections of either rIFN-γ alone (T1 *versus* T2 and T1 *versus* T3) or i.p. rIFN-γ followed by i.p. rIL-2 (T1 *versus* T4) resulted in increases in the proportions of tumor cells that stained positively for HLA class I and for HLA class II. The density of HLA class I expression on these tumor cells also was higher at these times relative to T1 (Fig. 1). The intensity of HLA class II expression also was increased after i.p. rIFN-γ (T1 *versus* T2), but no significant effect was found after the third injection of i.p. rIFN-γ (T3) or after rIL-2 (T4).

Production of TIL-derived T-Cell Lines for Adoptive Immunotherapy. Experiments were conducted to determine whether i.p. rIFN-γ or the combination of i.p. rIFN-γ and/or IL-2 facilitated the *in vitro* production of TIL-derived T-cell lines exhibiting primarily specific activity against autologous tumor cells. Peritoneal exudate cells were obtained on day 12 of the first cycle, and TIL-derived T-cell lines were produced from 3 patients. The phenotype and cytotoxicity are shown in Table 2. The T-cell line developed from the peritoneal exudate cells of patient 1 exhibited substantial cytotoxicity against autologous tumor cells. Attempts to grow TILs from specimens taken from patients at different stages of a treatment cycle produced the following results: pretreatment, 0 of 7; day 10 (after 3 rIFN-γ injections), 0 of 18; day 12 (after 3 rIL-2 injections), 3 of 18; and day 14 (3 days after the last dose of rIL-2), 0 of 12. The three successful cultures were developed from 8 patients from whom there had been a previous attempt to grow T-cell lines *ex vivo* in rIL-2 but without *in vivo* priming with i.p. rIFN-γ/rIL-2. Cultures that failed to produce T-cell lines comprised large numbers of adherent, mostly $CD3^-CD56^+$ cells (data not shown). Attempts to grow cells from nine patients after a second treatment course also was unsuccessful.

Table 2 Characteristics of TIL-derived T-cell lines produced in three patients after i.p. injections of rIFN-γ and rIL-2

	Patient 1	Patient 2	Patient 3
No. of cells harvested	3.01×10^{10}	1.96×10^{10}	2.86×10^{9}
MNL:tumor cell ratios	8:1	1:1.2	1:1.2
Phenotype (% positive)			
$CD3^+$	99	100	100
$CD4^+$	98	96	89
$TCR\alpha\beta^+$	96	100	88
$CD16^+$	1	2	2
$CD56^+$	5	21	6
$CD25^+$	72	19	75
$CD45RO^+$	80	37	89
In vitro cytotoxicity (%)			
Autologous tumor cells			
50:1	24.1	20.4	nd[a]
25:1	18.2	9.8	nd
12.5:1	21.4	7.2	nd
6.25:1	13.7	7.8	nd
Daudi cells			
50:1	14.3	3.5	24.1
25:1	8.1	2.5	21.6
12.5:1	8.3	1.2	11.0
6.25:1	4.1	2.2	16.4
K562 cells			
50:1	16.2	4.0	13.5
25:1	10.1	3.1	3.6
12.5:1	7.7	3.1	3.5
6.25:1	4.9	3.4	0.8

[a] nd, not done.

***In Vitro* Cytotoxicity of Peritoneal Effector MNLs Measured by ^{51}Cr Release.** i.p. rIFN-γ and rIL-2 resulted in significant increases in the cytotoxicity of peritoneal exudate cells at E:T ratios ranging from 6.25:1 to 25:1 (Fig. 2) at T4. *In vitro* cytotoxicity was increased significantly against allogeneic ovarian tumor cells ($P = 0.001–0.01$) as well as Daudi ($P = 0.0004–0.0017$) and K562 cells ($P = 0.0048–0.0064$) at all E:T ratios. At T3 after the third rIFN-γ dose, cytotoxic activity against Daudi cells was increased at all cell ratios, but cytotoxicity against K562 cells was increased only at higher E:T ratios ($P = 0.0738–0.0055$). Cytotoxicity against autologous tumor cells was detected after i.p. rIFN-γ plus rIL-2 in a few patients, but the differences between before and after treatment were not statistically significant. However, autologous cells used in these experiments contained variable numbers of tumor cells and mesothelial cells; lymphocytes and tumor cells had been separated from most specimens by using discontinuous gradients.

Modulation of MNL Subpopulations in Peritoneal Fluids after i.p. Therapy. Peritoneal fluid samples obtained before treatment contained large numbers of MNLs [mean ± SE, 236.65 ± 67.107 cells/mm^3 ($n = 20$)]. In 16 of the 20 specimens, two cell populations were identified on the basis of

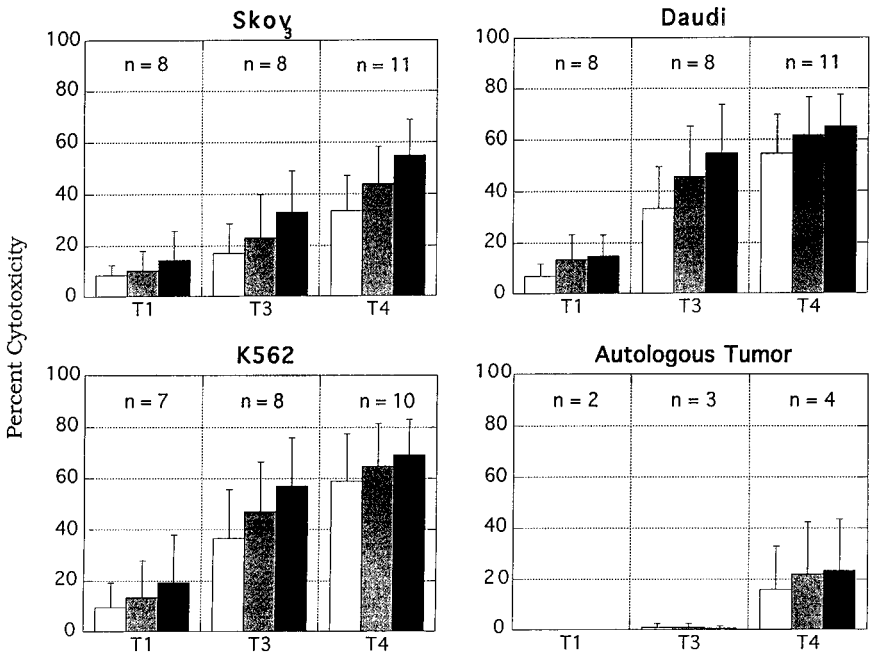

Fig. 2 Effect of i.p. rIFN-γ and rIL-2 on *in vitro* cytotoxic activity of peritoneal effector cells as measured by ^{51}Cr release. Results are shown for E:T ratios of 25:1, 12.5:1, and 6.25:1. *T1*, before treatment; *T3*, 3 days after the third rIFN-γ injection (day 10); *T4*, after all of the rIFN-γ and rIL-2 injections (day 12).

forward- and side-scatter characteristics. In one population of smaller cells, 91.7 ± 1.6% were LCA^+. In the second population of larger cells, only 11.3 ± 2.6% cells were LCA^+; the remainder were tumor and mesothelial cells. The total MNL population consisted mainly of $CD14^+$ cells (55.61 ± 5.28%) with or without $CD3^+$ cells (33.81 ± 4.46%). Additional analyses of the smaller cells revealed that >90% were MNLs, and most were T cells. Mean percentages of cells positive for surface antigens (± SE, n = 18–20) were as follows: $CD3^+$, 78.05 ± 2.93%; TCRαβ, 68.95 ± 3.44%; TCRγδ, 3.44 ± 0.5%; $CD4^+$, 44.05 ± 3.53%; $CD8^+$, 29.05 ± 2.53%; $CD16^+$, 8.58 ± 1.7%; $CD14^+$, 4.6 ± 0.9%; and $CD56^+$, 13.37 ± 1.94%. The CD4:CD8 ratio was 1.94 ± 0.33.

The effect of i.p. cytokine treatment was first examined in the total MNL population. Significant differences over time were detected in the total MNL cell counts, which increased from 236.65 ± 67.107 cells/mm^3 at T1 (n = 20) to 588.26 ± 125.01 cells/mm^2 at T2 (n = 19) after two doses of i.p. rIFN-γ (T1 *versus* T2, P = 0.0121). After i.p. rIFN-γ and rIL-2 (T4, n = 20) the cell count was 673.65 ± 205.24 cells/mm^3 (T1 *versus* T4; P, not significant). The Bonferroni test was used to correct for the large numbers of variables in the analysis (n = 38). With regard to proportions of T cells and monocytes, the mean proportion of total $CD3^+$ cells increased from 33.81 ± 4.46% (n = 16) before treatment to 54.69 ± 4.11% (n = 14) after all of the i.p. rIFN-γ and rIL-2 injections (T1 *versus* T4, P = 0.003). In contrast, the mean proportion of $CD14^+$ cells decreased from 55.16 ± 5.28% (n = 16) before treatment to 27.83 ± 4.30% (n = 15) after rIFN-γ plus rIL-2 (T1 *versus* T4, P = < 0.0005), which suggested that the numbers of T cells had increased relative to the numbers of monocytes during the treatment. Next we assessed the small cell population for differences in the proportions of $CD3^+$, $TCR\alpha\beta^+$, $TCR\gamma\delta^+$, $CD4^+$, $CD8^+$, $CD3^+$, or $CD16^+$ cells or in the CD4:CD8 ratio, but no treatment-related effects were detected (data not shown).

We then assessed the small-cell population for the presence of cells that expressed early ($CD69^+$), intermediate ($CD25^+$), or late (DR^+, $CD45RO^+$) activation antigens and naive T cells ($CD45RA^+$; Tables 3 and 4). The proportion of $CD56^+$ cells but not $CD3^+CD56^+$ cells (data not shown) increased from 13.37% ± 1.94% before treatment to 25.6 ± 2.60% at T3 after three i.p. rIFN-γ injections (P = 0.003) and to 25.42 ± 3.14% at T4 after rIFN-γ and rIL-2 (P = 0.04). Interestingly, the proportion of $CD25^+$ cells decreased from 18.07 ± 1.03% at T1 to 9.41 ± 1.12% at T3 (P = 0.008) to 9.79 ± 1.46% at T4 (P = 0.005). rIFN-γ i.p. or i.p. rIFN-γ and rIL-2 treatment was associated with increased proportions of $CD45RA^+$ cells (T1 *versus* T3, P = 0.011; T1 *versus* T4, P = 0.020) and $CD8^+CD45RA^+CDRO^+$ cells (T1 *versus* T2, P = 0.001), but no increases were detected overall in the $CD45RO^+$, $CD69^+$, or HLA-DR^+ cell populations. These findings suggest that proportions of naive T cells ($CD45RA^+$) increased relative to those of cells that expressed CD45RO, DR, or CD69 antigens, except for the bright $CD8^+CD45RO^+CD45RA^+$ subset, which had increased over the course of cytokine treatment. Proportions of $CD8^+DR^+$/total $CD8^+$ cells were 49.15 ± 4.81% before treatment and 61.05 ± 6.96% at T4 after both rIFN-γ and rIL-2. Although this increase was not statistically significant, the changes in the proportions of $CD8^+DR^+/CD8^+$ cells (late activation) *versus* $CD8^+CD69^+$/total $CD8^+$ (early activation) cells were significantly different (P = 0.007), which suggested that the proportions of $CD8^+DR^+$ cells were increased relative to those of the $CD8^+CD69^+$ population.

Expression of IL-2 and IFN-γ Transcripts before and during i.p. Therapy. Analysis of RNA extracts of peritoneal exudate cells from 13 patients showed a high frequency of

Table 3 Effects of i.p. rIFN-γ and rIL-2 on proportions of lymphocyte subsets that express early, intermediate, and late activation and antigens in peritoneal exudate[a]

Lymphocyte subsets (%)	T1	T2	T3	T4
$CD4^+CD45RO^+CD45RA^+$	1.84 ± 0.56 (14)	3.48 ± 0.66 (14)	2.66 ± 0.63 (14)	2.41 ± 0.55 (14)
$CD4^+CD45RO^-CD45RA^+$	5.24 ± 1.20 (14)	4.03 ± 0.69 (14)	5.01 ± 1.12 (14)	3.64 ± 0.74 (14)
$CD4^+CD45RO^+CD45RA^-$	30.54 ± 2.92 (14)	34.80 ± 3.44 (14)	27.99 ± 2.90 (14)	22.80 ± 3.40 (14)
$CD4^+CD45RO^+$/Total $CD4^+$	71.79 ± 5.42 (14)	80.93 ± 5.84 (14)	79.07 ± 4.54 (14)	76.50 ± 6.09 (14)
$CD8^+CD45RO^+CD45RA^+$[b]	2.14 ± 0.70 (14)	5.82 ± 0.89 (14)	5.01 ± 1.00 (14)	4.89 ± 0.87 (14)
$CD8^+CD45RO^-CD45RA^+$	11.77 ± 1.65 (14)	13.06 ± 1.63 (14)	14.19 ± 2.31 (14)	16.35 ± 2.37 (14)
$CD8^+CD45RO^+CD45RA^-$	15.65 ± 1.79 (14)	11.37 ± 1.12 (14)	10.84 ± 1.59 (14)	13.99 ± 2.02 (14)
$CD8^+CD45RO^+$/total $CD8^+$	46.93 ± 5.44 (14)	52.21 ± 4.97 (14)	47.21 ± 4.59 (14)	50.79 ± 4.82 (14)
Total $CD45RO^+$	49.34 ± 4.37 (14)	59.98 ± 4.53 (13)	49.33 ± 4.14 (14)	47.88 ± 5.26 (14)
Total $CD45RA^+$[b]	34.15 ± 3.34 (14)	44.87 ± 3.55 (14)	52.42 ± 3.49 (14)	49.21 ± 2.96 (14)
$CD4^+CD69^+$	3.49 ± 0.49 (11)	2.99 ± 0.87 (11)	2.57 ± 0.80 (11)	1.52 ± 0.11 (11)
$CD4^+CD69^-$	31.50 ± 4.15 (11)	41.68 ± 3.29 (11)	34.45 ± 3.79 (11)	27.07 ± 3.88 (11)
$CD4^+CD69^+$/total $CD4^+$	10.92 ± 1.92 (11)	6.43 ± 1.60 (11)	6.98 ± 1.87 (11)	6.81 ± 1.78 (11)
$CD8^+CD69^+$	12.31 ± 2.42 (11)	7.89 ± 1.05 (11)	8.83 ± 1.63 (11)	9.58 ± 1.40 (11)
$CD8^+CD69^-$	25.00 ± 3.21 (11)	28.00 ± 2.41 (11)	26.17 ± 4.38 (11)	33.48 ± 3.25 (11)
$CD8^+CD69^+$/total $CD8^+$[c]	32.41 ± 4.24 (11)	22.73 ± 3.06 (11)	25.68 ± 6.00 (11)	22.27 ± 2.69 (11)
Total $CD69^+$	24.01 ± 2.46 (11)	19.15 ± 2.38 (11)	30.55 ± 7.68 (11)	21.40 ± 2.62 (11)
$CD4^+HLA^-DR^+$	15.00 ± 2.30 (11)	16.68 ± 2.88 (11)	14.09 ± 2.36 (11)	13.73 ± 2.74 (11)
$CD4^+HLA^-DR^-$	26.16 ± 3.30 (11)	31.73 ± 2.65 (11)	25.68 ± 2.55 (11)	22.55 ± 2.68 (11)
$CD4^+HLA^-DR^+$/total $CD4^+$	36.86 ± 3.84 (11)	33.35 ± 3.86 (11)	34.39 ± 4.65 (11)	36.83 ± 5.54 (11)
$CD8^+HLA^-DR^+$	17.81 ± 3.42 (11)	17.84 ± 2.42 (11)	19.84 ± 2.24 (11)	24.88 ± 3.82 (11)
$CD8^+HLA^-DR^-$	13.20 ± 2.37 (11)	16.60 ± 2.92 (11)	16.40 ± 3.03 (11)	15.15 ± 2.73 (11)
$CD8^+HLA^-DR^+$/total $CD8^+$[c]	49.15 ± 4.81 (11)	53.15 ± 6.18 (11)	57.01 ± 5.38 (11)	61.05 ± 6.96 (11)
Total HLA^-DR^+	40.99 ± 3.50 (11)	40.99 ± 4.57 (11)	48.28 ± 4.85 (11)	48.35 ± 5.86 (11)
$CD56^+$[b]	13.37 ± 1.94 (19)	18.58 ± 1.69 (19)	25.61 ± 2.60 (18)	25.42 ± 3.14 (19)
$CD25^+$[b]	18.07 ± 1.83 (14)	13.52 ± 1.65 (14)	9.41 ± 1.12 (12)	9.79 ± 1.46 (14)

[a] Values are means ± SE, with number of patients in parentheses.
[b] Proportions with significant differences over time.
[c] Slopes for these subsets were significantly different ($P = 0.007$); T1, before treatment; T2, 2 days after the second rIFN-γ injection; T3, 3 days after the third rIFN-γ injection; T4, after all rIFN-γ and rIL-2 injections.

Table 4 Proportions changed over time (T1–T4) after Bonferroni adjustment for multiple comparisons

	P for pairwise comparison after Bonferroni adjustment					
Lymphocyte subsets	T1–T2[a]	T1–T3	T1–T4	T2–T3	T2–T4	T3–T4
$CD56^+$	0.394	0.003	0.040	0.013	0.125	1.000
$CD25^+$	0.031	0.008	0.005	1.000	0.395	1.000
Total $CD45RA^+$	0.049	0.011	0.020	0.227	0.506	1.000
$CD8^+CD45RO^+CD45RA^+$	0.001	0.034	0.056	1.000	1.000	1.000

[a] T1, before treatment; T2, 2 days after the second rIFN-γ injection; T3, 3 days after the third rIFN-γ injection; T4, after all rIFN-γ and rIL-2 injections.

transcripts for IL-2 (11 of 13 patients, including 4 of 5 ascites samples). In contrast, transcripts for IFN-γ were present less often (4 of 13 patients, including 1 of 4 ascites samples), which agrees with previous findings (8). IL-2 protein was detected in 10 of 18 samples and IFN-γ protein in 7 of 18 samples (data not shown). At T2 after i.p. rIFN-γ, IL-2 transcripts were detected in 12 of 12 patients, including 4 of 4 ascites samples, and IFN-γ transcripts were detected in 7 of 12 patients, including 3 of 4 ascites samples. Finally, at T4 after i.p. rIFN-γ and rIL-2, transcripts for IL-2 were detected in all of the specimens (12 of 12 patients, including 5 of 5 ascites samples), and transcripts of IFN-γ were detected in most specimens (10 of 12 patients, including 4 of 4 ascites samples).

Peritoneal Fluid Concentrations of IL-10 and TGF-β2 before and during i.p. Therapy. To determine the effect of rIFN-γ and rIL-2 on IL-10 and TGF-β2 peritoneal fluid concentrations, we determined by ELISA the concentrations of these cytokines on pretreatment peritoneal fluid specimens collected before and during the treatment, as shown in Fig. 3. The peritoneal IL-10 concentrations after i.p. injections of rIFN-γ were decreased in six, increased in three, and showed no change in nine patients. The *P* value using the Wilcoxon signed rank test for changes between peritoneal IL-10 concentrations observed before treatment (T1) and after 2 i.p. injections of rIFN-γ (T2) were only of borderline significance ($P = 0.055$). In contrast, peritoneal IL-10 concentrations were increased in 15 patients, decreased in 3, and showed no change in 1 patient after i.p. injections of rIL-2 (T4). The *P* values for changes in peritoneal IL-10 concentrations observed before i.p. rIFN-γ (T1) and after i.p. rIL-2 (T4) for all of the patients were significant ($P = 0.0293$). The *Ps* for the T1/T4 comparison were significant at the higher i.p. rIL-2 dosing ($P = 0.0l6$). In con-

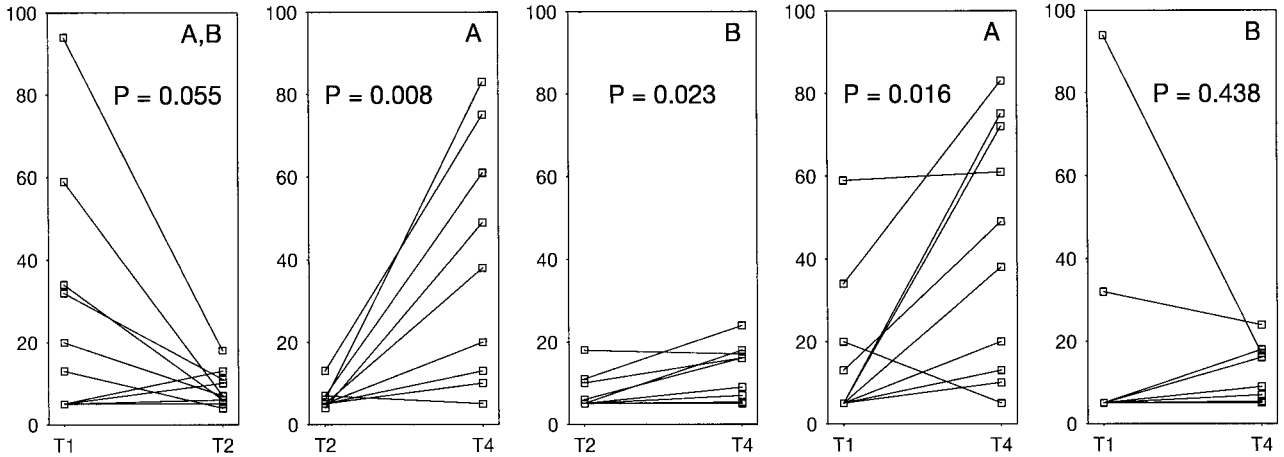

Fig. 3 Changes in peritoneal concentrations of IL-10 during i.p. rIFN-γ and rIl-2 treatment. Group A was given rIL-2, 6.0×10^5 IU/m^2, as a bolus dose; group B was given rIL-2, 1.0×10^5 IU/m^2, as a 6-h infusion. *T1*, before treatment; *T2*, 2 days after the second rIFN-γ injection (day 5 on Fig. 1); *T4*, after all of the rIFN-γ and rIL-2 injections (day 12). Analysis groups: *A*; *B*; *A,B*.

trast, i.p. injections of rIL-2 at the low dosing schedule were not associated with a significant change in peritoneal IL-10 concentrations ($P = 0.438$). Comparisons of changes in peritoneal IL-10 concentrations for all of the patients between after i.p. rIFN-γ (T2) and after i.p. rIL-2 (T4) were significant ($P = 0.0002$), after either the higher rIL-2 dosing ($P = 0.008$) or after the lower rIL-2 dosing ($P = 0.023$).

Ten of 19 pretreatment peritoneal fluid specimens had detectable TGF-β2 by ELISA. The mean ± SE was 368 ± 240 pg/ml before treatment, 321 ± 259 pg/ml after two injections of rIFN-γ, and 323 ± 202 pg/ml after rIFN-γ and rIL-2. Mean TGF-β2 concentrations did not change over the three measurement periods.

Effects of i.p. rIFN-γ and i.p. rIL-2 on Peritoneal Fluid Concentrations of Neopterin and Nitrate. Neopterin and nitric oxide were measured as products of activated macrophages. Before the treatment (T1) the mean neopterin level was 6.4 ± 1.64 μmol/liter ($n = 20$) and nitrate 28.93 ± 4.55 μg ($n = 16$). After two doses of i.p. rIFN-γ, the neopterin concentration increased to 22.84 ± 3.93 μmol/liter ($n = 20$; $P = .0006$), and nitrate increased to 55.91 ± 14.16 μg ($n = 20$; $P = .094$). After i.p. rIFN-γ and rIL-2, neopterin increased to 27.38 ± 3.96 μmol/liter ($n = 20$; $P = 0.0003$) and nitrate to 47.27 ± 5.96 μg ($n = 16$; $P = 0.0216$).

DISCUSSION

i.p. rIFN-γ combined with i.p. rIL-2 at bolus doses of 6.0×10^5 IU/m^2 (group A) or as a 6-h infusion at a dose of 1.0×10^5 IU/m^2 (group B) was tolerated overall. More serious nonhematological side effects occurred in only 11.6% of courses. The percutaneous catheters used in this study are a convenient and relatively inexpensive method for i.p. administration, even for those patients who do not have ascites. Adverse events requiring replacement occurred in 6 (27%) of 22 patients, similar to the experience of others with different catheters. The advantages of percutaneous catheters over implanted devices include the ease with which these catheters can be placed or removed (2). Using the same serum-free conditions that were used previously for the *ex vivo* development of ovarian TIL-derived T-cell lines for adoptive therapy (4, 26), TIL-derived T-cell lines that were mostly CD4$^+$ were produced in only 3 (15%) of 20 patients in this series. Specimens from three additional patients were expanded up to but not beyond the selection phase. The low frequency of production of TIL-derived T-cell lines from patient samples after i.p. priming with rIFN-γ/rIL-2 could be attributed to several factors that are discussed below.

rIFN-γ i.p. either alone or in combination with rIL-2 i.p. after rIFN-γ increased both the proportions of HLA class I$^+$ and HLA class II$^+$ tumor cells in the peritoneal cavity and the density of expression of these antigens. Tumor cells that do not express HLA class I or II antigens cannot be recognized by effector cytotoxic lymphocytes and cannot elicit effective MHC-restricted immune responses. rIFN-γ i.p., either alone or in combination with rIL-2 after rIFN-γ, augmented the expression of HLA class I and class II. rIL-2 alone does not seem to affect the expression of MHC antigens on tumor cells, although the direct contribution of rIL-2 in enhancing MHC expression, when administered in combination with rIFN-γ, is unclear. Nevertheless, rIL-2 would not be expected to have a direct effect on tumor cells and enhance either HLA class I or II expression, because ovarian tumor cells do not express any IL-2 receptors. However, because tumor cells usually lack costimulatory antigens, other antigen-presenting cells may be necessary both for antigen presentation and for the costimulatory signals needed for specific T-cell activation *in vivo*. We recently have demonstrated a population of dendritic cells in the peritoneal cavity of patients with ovarian carcinoma (30). These cells have an immature phenotype and have low or absent expression of CD80 and CD11c (30). Moreover, we could not detect IL-12 production in the peritoneal cavity of these patients (30). It is presently unknown whether these cells can undergo functional maturation *in vivo*. In two ongoing clinical trials, we are attempting to determine whether i.p. rhIL-12 facilitates T-cell activation without IL-10 production *in vivo* (31) and whether B7-transduced

ovarian autologous tumor cells may provide effective costimulation *in vivo* (32).

We and others have shown that T cells obtained from the peritoneal cavity of patients with EOC exhibit characteristics of *in vivo* activation (8). These cells express certain early, intermediate, and late activation antigens (Table 3). In the present study, the transcript for both IL-2 and protein were present in most peritoneal exudate specimens; a smaller proportion of specimens also were positive for both IFN-γ transcript and protein, results that are in agreement with our previous findings (8). Because rIFN-γ followed by rIL-2 may stimulate the proliferative and the cytotoxic responses of $CD8^+$ T *cells in vitro* (22), we hypothesized that this combination of cytokines would facilitate the priming of T cells *in vivo.* Indeed this treatment increased the proportions of $CD8^+DR^+$/total $CD8^+$ (late activation) relative to that of $CD8^+CD69^+CD4^+$/total $CD8^+$ (early activation) cells. These findings are generally consistent with the *in vitro* findings of Siegel (18), who has shown that the proportions of $CD8^+DR^+$ T cells are increased after *in vitro* treatment with rIFN-γ followed by rIL-2. Our observation that the proportions of double-bright $CD8^+CD45RA^+CD45RO^+$ cells increased significantly after i.p. rIFN-γ also suggests that these cells have been stimulated directly or indirectly by the injected cytokines. Possibly, these cells express a transitional phenotype before losing the $CD45RA^+$ antigens as a result of activation. In contrast, the finding that the proportions of $CD25^+$ cells decreased significantly after i.p. treatment with rIFN-γ alone or i.p. rIFN-γ combined with rIL-2 might indicate either loss or internalization of the receptor. In a previous study, i.p. rIL-2 alone produced an increase in peritoneal-cavity soluble-IL-2-receptor concentration (24). In the present study, i.p. rIL-2 preceded by i.p. rIFN-γ increased the proportions of $CD45RA^+$. Because $CD45RA^+$ cells are a naive cell population, the increase in the proportions of these cells could suggest migration of such cells into the peritoneal cavity from other sites such as the blood. Moreover, the proportions of peripheral blood $CD45RA^+$ lymphocytes relative to $CD45RO^+$ cells are higher than in peritoneal exudate cells (data not shown). The increases in the proportion of $CD56^+$ cells after i.p. rIFN-γ paralleled the increase in peritoneal effector cell-mediated cytotoxicity against target cells from hemopoietic tumor cell lines, and the presence of TGF-β2 and IL-10 did not seem to interfere with this cytotoxicity (data not shown).

IL-10 and TGF-β are important immunosuppressive cytokines, and both have been identified in patients with ovarian cancer. IL-10 concentrations in peritoneal fluids were increased after higher i.p. rIL-2 dosing of 6.0×10^5 IU/m^2. IL-10 concentrations were not increased after i.p. rIFN-γ alone, although neopterin levels, which reflect macrophage activity, were increased both after i.p. rIFN-γ and after the combination of i.p. rIFN-γ/rIL-2. Recently, we have identified an ovarian ascitic macrophage that has the DR^- phenotype as a primary producer of IL-10 (33). In other *in vitro* experiments,[5] we have shown that rIFN-γ and TNF-α suppresses IL-10 production by these DR^- macrophages. In a previous clinical trial (4), we showed that i.p. rhIL-2 alone at 6×10^5 IU/m^2 daily for 4 days resulted in increased peritoneal fluid IL-10 concentration in four of four patients. Taken together, these findings suggest that i.p. rIL-2 at the higher bolus dosing and, even without the addition of i.p. rIFN-γ, could be an important contributor to the increased concentration of IL-10. There is also the possibility that $CD14^+DR^-IL\text{-}10^+$ macrophages could be responsible for the production of IL-10 in these patients. Increased levels of TGF-β2 were detected in >50% of peritoneal fluid specimens before treatment. Unlike IL-10, no significant changes were detected during the treatment with i.p. rIFN-γ/rIL-2. TGF-β isotypes are produced by different cell populations associated with EOC, and include both tumor cells (27, 34) and monocytes (33) and lymphocytes (34).

Increased production of nitric oxide could be another important factor that could interfere with T-cell activation in EOC. Significant nitrate levels were detected in pretreatment specimens, and levels increased after either i.p. rIFN-γ or i.p. rIFN-γ/rIL-2. Future research should examine the possibility of reducing nitric oxide production or its effects, after treatment with cytokines that specifically increase its production.

[5] Unpublished results.

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