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Local antitumour treatment in carcinoma patients with bispecific-monoclonal-antibody-redirected T cells

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Abstract. In a pilot clinical study carcinoma patients with malignant ascites or pleural exudates have been treated locally with autologous lymphocytes activated ex vivo and redirected towards tumour cells with bispecific monoclonal antibodies. BIS-1, the bispecific monoclonal antibody used in this study, combines specificity against a tumour-associated antigen, AMOC-31, present on carcinomas, with a specificity against the CD3 complex on T lymphocytes. Patients selected for treatment had malignant pleural or peritoneal effusions. Treatment consisted of isolating autologous peripheral blood lymphocytes, ex vivo activation, incubation with bispecific monoclonal antibodies and injection at the effusion site of these BIS-1-redirected lymphocytes. To evaluate the effects of the bispecific monoclonal antibody, five patients received treatments with activated lymphocytes without bispecific antibodies. Effusion samples taken before and at various times after treatment were analysed by immunocytology and for the presence of the soluble factors carcinoembryonic antigen (CEA), interleukin-6 (IL-6), tumour necrosis factor (TNF), C-reactive protein and soluble CD8. In this way both immune activation and anti-tumour activity could be monitored. Conjugate formation between tumour cells and activated lymphocytes was seen as soon as 4 h after injection of BIS-1-redirected activated lymphocytes, followed by a disappearance or reduction of tumour cells after 24–48 h. In parallel with this, the soluble tumour marker CEA decreased in the effusion fluid following injection with the BIS-1-redirected lymphocytes. Furthermore, a steep increase in local granulocyte numbers was observed in the effusion fluid, which reached a maximum 24–48 h after the start of the treatment. Also levels of IL-6 and TNF were greatly elevated. The data suggest that the treatment induces both antitumour activity and a strong local inflammatory reaction. This is accompanied by no or only minor local and systemic toxicity, i.e. mild fever, which disappeared as the local inflammatory reaction diminished 48–72 h after treatment.

Key words: Immunotherapy – Bispecific monoclonal antibodies – T cell targeting – Inflammation – T cell activation – Carcinoma

Introduction

Adoptive cellular and humoral immunotherapy have proven to be effective treatments in a number of melanoma and renal cell carcinoma cases. However, both the application of lymphokine-activated killer cells and the administration of monoclonal antibodies (mAb) have been shown to have only limited applicability in inducing antitumour responses in other tumour types [3, 25, 26, 41]. A novel approach is to combine the specific recognition properties of monoclonal antibodies with the killing capacity of activated cytotoxic T lymphocytes [2]. Bispecific monoclonal antibodies (bsmAb), combining the specificities of two monoclonal antibodies in one molecule, may serve such a purpose since they are able to recognize and bind both to tumour-associated membrane antigens on tumour cells and to receptors on cytotoxic cells [7, 18, 22, 33, 34]. This induces a functional linkage between effector and target cells, enabling specific recognition and killing of the target cells by the effector cells. Indeed bsmAb have been shown to be useful in focusing effector cell populations provided that triggering receptors present on the effector cells are involved in the bsmAb-mediated effector/target interaction. Antitumour activity using bsmAb has been shown in vitro and in vivo in mouse models for various effector cells and many different target cells [5, 6, 8, 17, 19, 20, 40]. Until now, one clinical pilot study using the bsmAb approach has been described for a group of glioma patients receiving local, intrathecal, treatment with effector cells

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activated ex vivo in combination with a bispecific antibody directed against both CD3 and neural cell adhesion molecule (NCAM) [21]. Furthermore, safe intraperitoneal administration of bsmAb directed against both CD3 and the folate receptor, which is overexpressed by ovarian carcinomas, has been reported [16]. The bsmAb used in the present study reacts with a pan-carcinoma-associated membrane antigen called AMOC-31 and the CD3 complex present on T lymphocytes. The antigen AMOC-31 is a 40-kDa membrane-bound glycoprotein expressed by most carcinomas. This antigen has been described extensively and is also recognized by a number of mAb including AUA-1 and CO17-1A [10, 32]. As AMOC-31 is strongly expressed on most carcinoma tumours, and is not shed into the circulation [42], it appears to be a good target structure for bsmAb-redirected cytotoxicity. In the present report, we describe a study on the effect of bsmAb-targeted T lymphocytes applied to patients suffering from malignant ascites or pleural effusion. The patients received local treatments with autologous T lymphocytes activated ex vivo in the presence or absence of bsmAb. One patient was studied more extensively and is presented in detail.

Materials and methods

Selection of patients

Patients treated had histologically proven malignancy, not susceptible to any form of conventional therapy. Their median age was 48 years (range 45–73). All patients had symptoms of this effusion not controllable by frequent draining. All patients had an ECOG performance score of 3. Inclusion criteria for treatment were the presence of ascites or pleural effusion containing AMOC-31-positive tumour cells. The treatment protocol was approved by the Hospital Medical Ethical Committee and informed consent was obtained from the patients. Patients included in the study and the number and form of the treatments are listed in Table 1. Patient 9 was treated most extensively and is described in more detail.

Treatment protocol

Autologous lymphocytes were isolated by density centrifugation (Lymphoprep, Nycomed Norway) at 2400 rpm for 20 min 5 days prior to treatment. After two washings in RPMI-40 medium (Gibco Europe, Breda, The Netherlands) and centrifugation at respectively 1800 rpm and 1200 rpm for 10 min, a minimum of 1.0×10^8 cells were resuspended in culture medium at a final concentration of 0.5×10^6/ml. Culture medium consisted of RPMI-40 medium supplemented with 2% heat-inactivated human pool serum, 2 mM glutamine, 60 μg/ml gentamicin (Schering, Kenilworth, USA) and 5% v/v WT-32 hybridoma culture supernatant. WT-32 is a monoclonal antibody directed against the ε chain of the CD3 complex and has been shown to be a highly mitogenic antibody [24, 36]. After 3 days, the cells were washed once by centrifugation at 1000 rpm for 10 min and resuspended in fresh culture medium supplemented with 60 IU/ml recombinant IL-2 only. After culturing for 2 additional days, cells were harvested by centrifugation and resuspended in 5 ml 0.9% NaCl, 0.5% human serum albumin (Institut Merieux, Lyon, France). The cell viability of the suspension, assessed light microscopically by trypan blue exclusion, was always above 90%. The cells were then incubated with or without bsmAb at a final concentration of 0.1 mg/ml at 0° C for 30 min, 20 ml 0.9% NaCl, 0.5% human serum albumin was added to give a final volume of 25 ml and the cell/antibody mixture was injected slowly either intraperitoneally or intrapleurally at the site of the effusion. Sterility of the injected cell/antibody mixture was tested by culturing a sample of the end-product at 27° C and 37° C. In one case, patient 9, 7500 IU interleukin-2 (IL-2; Eurocetus, Amsterdam, The Netherlands) was administered at the effusion site 24 h and 48 h after injection. In addition, during treatment with bsmAb, this patient received an extra 350 μg BIS-1 bsmAb together with the IL-2. A summary of each treatment is given in Tables 1, 2.

Evaluation

The treatment was evaluated by analysis of soluble factors present in blood plasma and effusion fluid as well as by immunocytological analysis of effusion samples. Samples were taken just prior to and 4, 24, 48, 72 h, 6 days and 2 weeks after injection of the lymphocytes activated ex vivo.

Evaluation of soluble factors. For blood plasma and effusion fluid analysis, samples were drawn into EDTA-containing glass tubes (Becton Dickinson, Mountainview, Calif., USA). Directly after the samples has been taken, they were placed on ice, centrifuged at 4° C, 2500 rpm for 10 min, and the clear supernatants were subsequently stored at –20° C. The samples were analysed for the presence of carcinoembryonic antigen (CEA), interleukin-6 (IL-6), tumour necrosis factor (TNF), C-reactive protein (CRP), and soluble CD8 (sCD8). TNF was measured by radioimmunoassay (Centocor Europe, Leiden, The Netherlands). CEA (Abbott, Belgium), CRP [31], IL-6 [9] and sCD8 (T-Cell Sciences, Cambridge, USA) were assessed by ELISA. Human anti-(mouse Ig) antibodies that could interfere with the mouse-mAb-based sCD8 ELISA were removed from the samples by protein G (Pharmacia, Uppsala, Sweden) adsorption.

Immunocytological analysis. For immunocytological analysis approximately 10 ml effusion fluid was collected in heparinized glass tubes (Becton Dickinson, Mountainview, California, USA), which were subsequently centrifuged at 1000 rpm for 10 min. Cell pellets were suspended in 3 ml phosphate-buffered saline (PBS) and cells were spun down in a cyto-centrifuge (Shandon, Astmoor, England) at 500 rpm for 5 min. Cytospin preparations were air-dried for a minimum of 30 min and fixed in acetone for 10 min. They were then air-dried and immediately immunostained with MOC-31 [31] to evaluate the presence of tumour cells and CD45 to evaluate the presence of leucocytes. In addition, May Grunwald Giemsa (MGG) staining was performed for additional cytological evaluation. To evaluate ICAM-1 expression on tumour cells, double-fluorescence immunostaining was performed with CD54 (IgG2) and MOC-31 (IgG1) followed by isotype-specific rhodamine-isothiocyanate(RITC)- and fluorescein-isothiocyanate(FITC)-labelled goat anti-(mouse IgG1) and anti-(mouse IgG2) antibodies. The preparations were embedded in Citifluor (Citifluor Ltd., London, UK) and evaluated using fluorescence microscopy. Horseradish-peroxidase-stained preparations were all counter-stained with haematoxylin solution, embedded in mounting medium and evaluated by light microscopy.

Antibodies. For immunostaining, horseradish-peroxidase-labelled goat anti-(mouse Ig) (Dako, Glostrup, Denmark), IgG1- and IgG2-isotype-specific RITC- and FITC-labelled goat anti-(mouse Ig) (Southern Biotechnology Associates, Birmingham, USA), CD54 (Becton Dickinson, Birmingham, USA, IgG2b) and CD45 (MCA development, Groningen, The Netherlands) were used 1 : 20 diluted in PBS. MOC-31 was used as undiluted hybridoma culture supernatant. For lymphocyte activation, WT32 (IgG2a), a mitogenic CD3 antibody (kindly provided by W. Tax [36]) was used. Addition of 5% (v/v) hybridoma culture supernatant resulted in maximal mitogenic activity [24].

T cell targeting. The hybrid hybridoma producing the bispecific monoclonal antibody BIS-1 was made by fusion of the hybridomas MOC-31 (IgG1) and RIV-9 (IgG3), which recognize AMOC-31 on carcinoma cells and CD3 on T cells respectively. The hybridoma RIV-9 was a kind gift of Dr. H. Kreeftenberg (RIVM, Bilthoven, The Netherlands). The

Table 1. Characteristics and treatment episodes of the patients included in the study

Patient	Age (years)	Sex	Carcinoma typed as	Site of effusion	Treatment given[a]
1	57	M	Colon ca.	Peritoneum	AT
2	52	F	Mamma ca.	Peritoneum	AT
3	64	F	Ovarian ca.	Peritoneum	RAT/RAT
4	48	M	Lung ca.	Pleura	RAT[b]
5	73	F	Lung ca.	Pleura	RAT
6	47	M	Colon ca.	Peritoneum	AT/RAT
7	47	F	Ovarian ca.	Peritoneum	AT/RAT
8	52	M	Colon	Peritoneum	AT/RAT
9	45	F	Gastric ca.	Peritoneum	RAT/AT/RAT/AT

[a] AT, activated T lymphocytes; RAT, redirected activated T lymphocytes
[b] Patient died after treatment

hybrid hybridoma was made as described [12]. In short, a hypoxanthine guanine phospho ribosyl transferase (HPGRT)-negative MOC-31 hybridoma cell line was selected by culturing the hybridoma cell line in 8-azaguanine-containing medium. This hybridoma cell line, unable to grow in hypoxantine, aminopterine and thymidine (HAT) medium, was made neomycin-resistant by retroviral transfection. The HAT-sensitive, neomycin-resistant MOC-31 hybridoma cell line was then fused with the HAT-resistant neomycin-sensitive hybridoma cell line RIV-9 using standard polyethyleneglycol fusion procedures and plated in a 96-well culture plate. Hybrid hybridomas were selected by culturing the HAT/neomycin-containing medium. One hybrid hybridoma, producing high amounts of bsmAb (BIS-1) was selected and cloned. Purification of the bsmAb antibody from culture supernatant was performed by protein A (Pharmacia, Uppsala, Sweden) column chromatography. The different antibody fractions were eluted from the column using a pH gradient. The purified bispecific antibody (IgG1/IgG3) was then dialysed against 0.9% NaCl; 0.5% human serum albumin was added and the solution was sterilised by passing it through a 0.22-μm filter. Sterility was checked by culturing a sample at 27° C and 37° C and pyrogen contamination was excluded using a *Limulus* amoebocyte lysate assay.

Results

General

In Table 1, some characteristics of the patients enrolled in the study are given including the site and kind of treatment. Two patients were treated intrapleurally, and seven intraperitoneally. Two patients received a single treatment with activated T lymphocytes only (AT treatment). Three patients received one or more treatments with bsmAb-redirected activated T lymphocytes (RAT treatment), and four patients received two or more treatments with activated T lymphocytes to which bsmAb were or were not added. Patient 9 received four treatments with activated T lymphocytes in the presence or absence of bsmAb. The effector cells used for reinfusion resulted from a 5-day in vitro culturing protocol designed to activate and expand T cells preferentially as was shown by immunostaining against CD3 (more than 95% positive) and CD8 (more than 70% positive). When used in a ^{51}Cr-release assay, T cells activated by this protocol were shown to possess a high specific lytic capacity against relevant tumour cells (data not shown). In Table 2, the most prominent results obtained with immuno- and MGG stainings are summarized. After treatment in the presence of bsmAb (RAT treatment), increased numbers of granulocytes were detected at the site of treatment in all patients 24–48 h after reinfusion of the immune cells. The same applies for the presence of tumour/lymphocyte conjugates after 1–4 h, and the presence of dead tumour cells 24 h after reinfusion of the immune cells. All these phenomena were observed in MGG and immunocytologically stained cytospin preparations made from effusion samples. Clinical symptoms of toxicity were mainly due to insertion of the intraperitoneal catheter. In addition low-grade fever (grade 1 WHO) was observed in almost all patients. Treatment with activated T lymphocytes only (AT treatment), resulted in few side-

Table 2. Treatment and immunocytological evaluation of the treatment given

Patient	Treatment given[a]	Number of immune cells injected	Granulocyte influx	Tumour-lymphocyte conjugation	Tumour cell lysis	Side-effects
1	AT	7×10^7	–	–	–	–
2	AT	2×10^7	–	–	–	–
3	RAT	1×10^8	+	+	+	–
3	RAT	1.5×10^8	+	+	+	–
3	RAT	1.5×10^8	+	+	+	–
4	RAT	1.5×10^9	+	+	+	Fever[b]
5	RAT	5×10^8	+	+	++	–
6	AT	6×10^8	+	+/–	+/–	–
6	RAT	6×10^8	+++	+	++	–
7	AT	2.5×10^8	+	–	+/–	–
7	RAT	1×10^9	+++	+	+	Fever
8	AT	3.5×10^8	ND	ND	ND	–
8	RAT	3×10^8	ND	ND	ND	Abdominal pain
9	AT	6×10^8	+	+	+	–
9	RAT	6×10^8	+++	+	+++	Fever
9	AT	5×10^8	+/–	+	+	–
9	RAT	5×10^8	+++	+	+++	Fever

[a] AT, activated T lymphocytes; RAT, redirected activated T lymphocytes
[b] Patient died after treatment

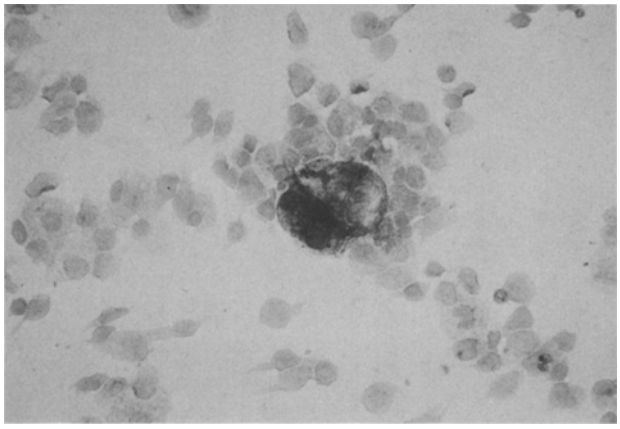

Fig. 1. Immunoperoxidase staining against AMOC-31 of an ascitic fluid sample taken from patient 9 4 h after injection with redirected activated T lymphocytes showing tumour cells (*t*) surrounded by leucocytes (l)

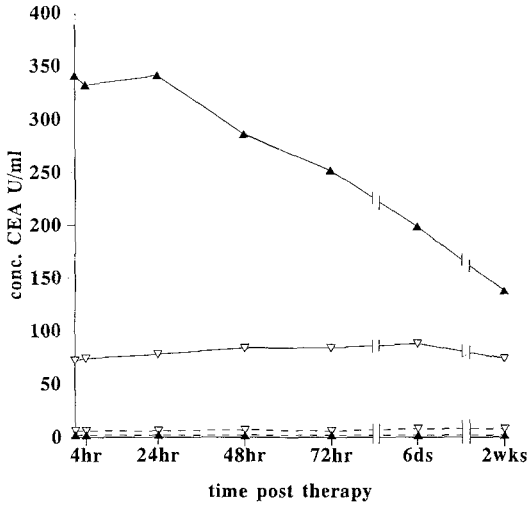

Fig. 2. Intraperitoneal (—) and systemic (- - -) concentrations of carcinoembryonic antigen (*CEA*) of patient 9 before and after intraperitoneal treatment with activated T lymphocytes in the presence (▲) and absence (▽) of bispecific mAb (bsmAb)

effects, which were similar to those described above. One patient (patient 8) complained of prolonged burning sensation in the abdomen after reinfusion of the lymphocytes. Granulocyte influxes as well as tumour/lymphocyte conjugates were seen to a limited extend in these AT-treated patients, and tumour killing was clearly less pronounced. To enable further determination of the effect of bsmAb on the treatment, in patient 9, additional parameters including CEA, TNF, IL-6 and CRP concentrations were assessed. This was done both locally from the site of treatment as well as systemically in serum at different times after treatment.

Patient 9

Antitumour response. Cytospin preparations of ascites samples taken from patient 9 before and after treatment with activated T cells alone and with activated T cells in the presence of bsmAb were immunostained with either

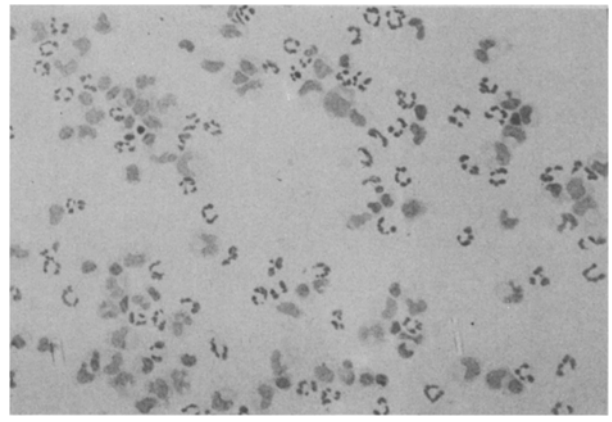

Fig. 3. May Grunwald Giemsa staining of an ascitic fluid sample taken rom patient 9 48 h after treatment with redirected activated T lymphocytes showing large amounts of granulocytes

MOC-31 or CD45 and analysed light-microscopically. As shown in Fig. 1, 4 h after reinjection of the redirected activated T cells, conjugates between tumour cells and activated lymphocytes were present; 24 h after injection, tumour cells could no longer be detected. This situation continued for 4 weeks. A substantial decrease in ascites volume was noticed during this period. Parallel to these observations, as shown in Fig. 2, the CEA concentration in the ascitic fluid, which was very high at the start of therapy (341 μg/l; normal serum value below 2.5 μg/ml) decreased upon treatment with retargeted activated T lymphocytes to 138 μg/ml 14 days after treatment. Another soluble-carcinoma-related tumour marker, TAG72.4, also decreased during this treatment (data not shown). The second treatment period, in which the patient received activated T lymphocytes without bsmAb, started 6 weeks after the first treatment. Tumour cells had returned into the ascites at that time, and the concentration of CEA in the ascitic fluid was, although lower than at the start of the first treatment, still higher than normal serum values. Upon treatment with autologous activated T lymphocytes in the absence of bsmAb, incomplete disappearance of tumour cells, as evaluated by immunocytology, was detected. Also the concentration CEA in the ascitic fluid remained stable. The serum concentration of CEA did not change notably during treatment in either the presence or absence of bsmAb.

Inflammatory reaction. With treatment in the presence of bsmAb, a steep increase in granulocyte number in the peritoneal cavity was seen. This increase in granulocyte number, as shown in Fig. 3, was most elevated 48 h after therapy. The granulocyte number had returned to normal values 6 days after the treatment, when macrophages had become the most prominent cell type. During treatment in the absence of bsmAb, granulocyte numbers also appeared to increase; however, this was much less prominent when compared to treatment in the presence of bsmAb. To investigate this inflammatory reaction further, ascites and blood samples were analysed for the presence of a number of inflammatory factors. The concentration of IL-6 (Fig. 4) as well as TNF (Fig. 5) was found to be increased considerably in ascites during treatment in the presence of bsmAb.

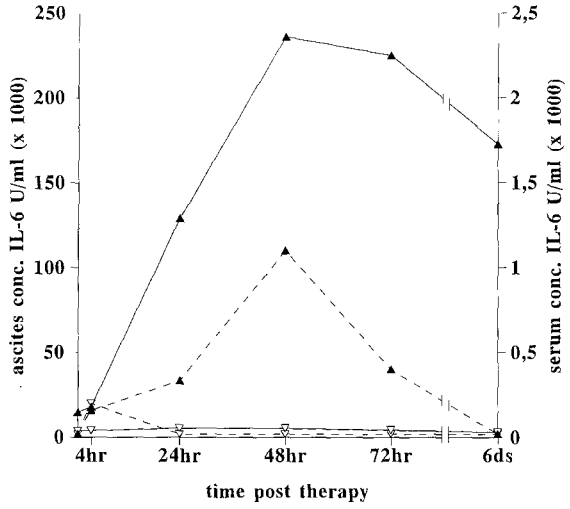

Fig. 4. Intraperitoneal (—) and systemic (- - -) concentrations of interleukin-6 (*IL-6*) of patient 9 after intraperitoneal treatment with activated T lymphocytes in the presence (▲) and absence (▽) of bsmAb

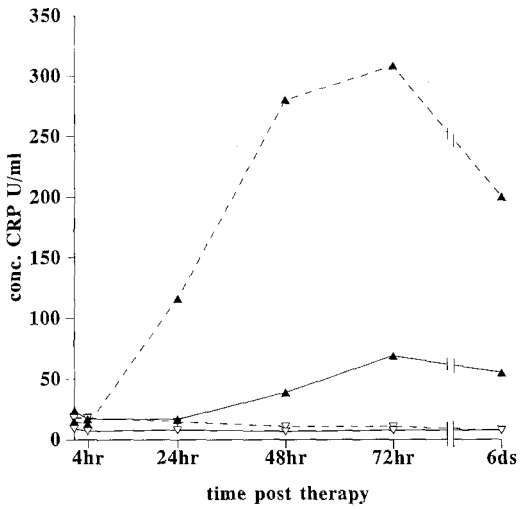

Fig. 6. Intraperitoneal (—) and systemic (- - -) concentrations of C-reactive protein (*CRP*) of patient 9 after treatment with activated T lymphocytes in the presence (▲) and absence (▽) of bsmAb

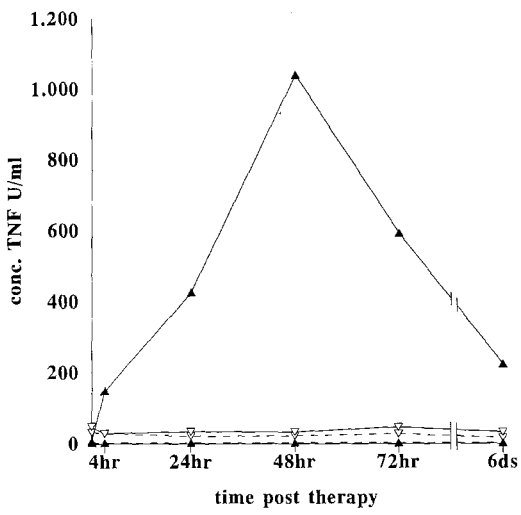

Fig. 5. Intraperitoneal (—) and systemic (- - -) concentrations of tumour necrosis factor (*TNF*) of patient 9 after intraperitoneal treatment with activated T lymphocytes in the presence (▲) and absence (▽) of bsmAb

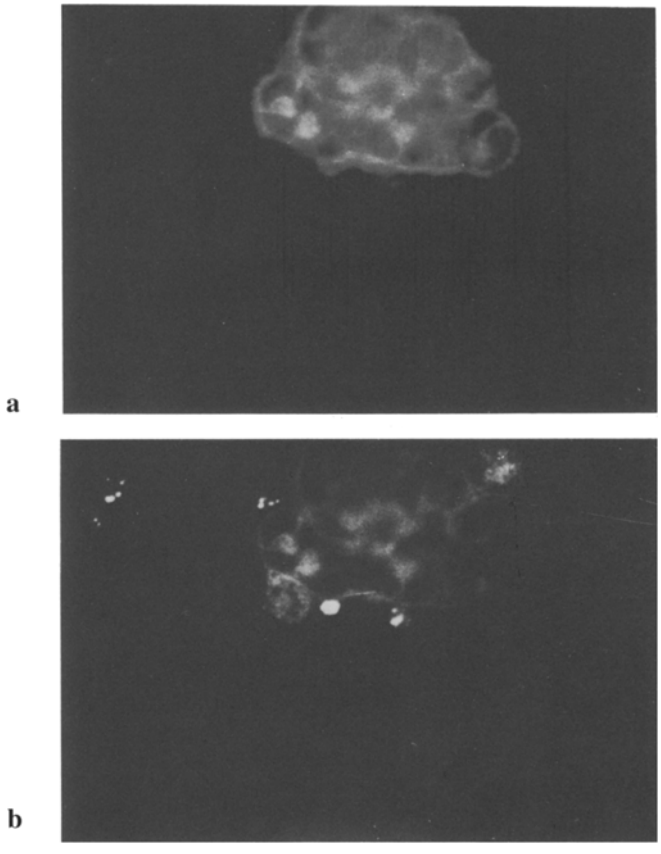

Fig. 7. Double-immunofluorescence staining against AMOC-31 (**a**; red fluorescence) and ICAM-1 (**b**; green fluorescence) of an ascitic fluid sample taken just prior to treatment with bsmAb-redirected activated T lymphocytes. As shown, AMOC-31-positive tumour cells are negative for ICAM-1 prior to the therapy

In contrast, during treatment in the absence of bsmAb, ascites concentrations of IL-6 and TNF remained stable. During treatment in the presence of bsmAb, IL-6 and CRP (Fig. 6) but not TNF were found to be increased in the blood serum after therapy. CRP increased to a peak concentration of 308 mg/l 72 h after therapy and appeared to be somewhat delayed compared to the local elevation of IL-6 and TNF. The bsmAb-mediated inflammatory reaction appeared to be also reflected in an up-regulation of ICAM-1 on the tumour cells as shown by immunofluorescence staining (Fig. 7, 8). During treatment in the absence of bsmAb, no up-regulation of ICAM-1 could be observed on the tumour cells.

T-cell activation status in vivo. Monitoring the T cell activation status of the injected T lymphocytes was performed by measuring sCD8 in the samples taken before and after therapy. As shown in Fig. 9, sCD8 increased markedly in the ascites during therapy in the presence of bsmAb from 245 U/ml to 1008 U/ml. During treatment without bsmAb, ascites sCD8 concentrations stayed within the normal range present in serum (400–600 U/ml). In

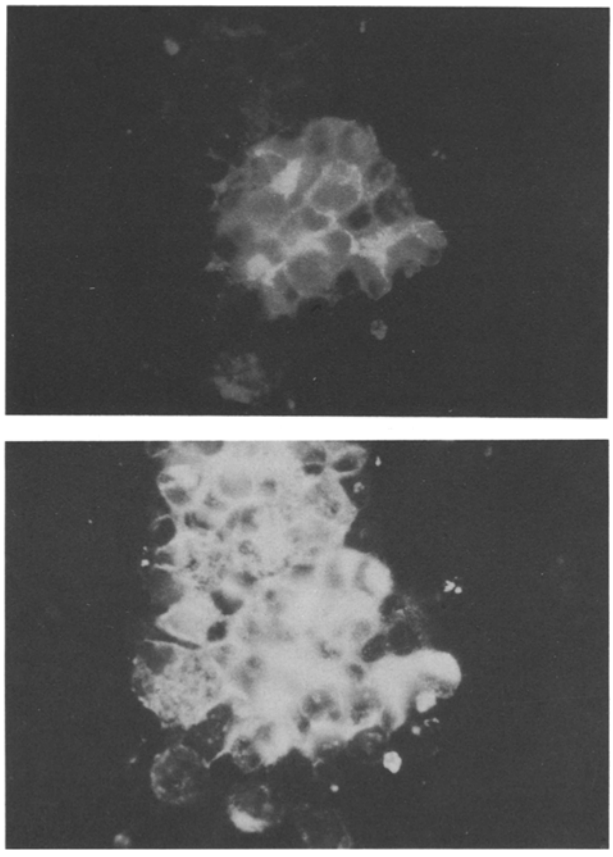

Fig. 8. Double-immunofluorescence staining against AMOC-31 (**a**; red fluorescence) and ICAM-1 (**b**; green fluorescence) of an ascitic fluid sample taken 4 h after treatment with bsmAb-redirected activated T lymphocytes. As shown, 4 h after injection of the bsmAb-redirected activated T lymphocytes, AMOC-31-positive tumour cells have become positive for ICAM-1

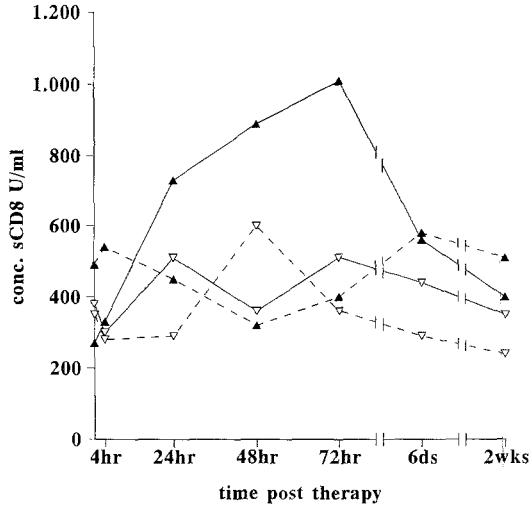

Fig. 9. Intraperitoneal (—) and systemic (- - -) concentrations of soluble CD8 (*sCD8*) of patient 9 before and after therapy, after treatment with activated T lymphocytes in the presence (▲) and absence (▽) of bsmAb

blood samples, sCD8 was found not to change notably during treatment in either the presence or absence of bsmAb.

Discussion

The present report deals with a first pilot study on local treatment of carcinoma patients with autologous activated and bsmAb-redirected T lymphocytes. The objective was to investigate the in vivo effects of bsmAb when used in a therapeutic setting for the treatment of cancer. A local treatment protocol was set up to circumvent a number of possible problems. First, treatment in body cavities obviates possible lymphocyte-to-tumour homing problems since the effector cells are directly injected at the site of the tumour. Furthermore, treatment in a "closed" compartment facilitates evaluation as post-therapy samples can easily be taken for analysis. Finally, toxic side-effects due to lysis of AMOC-31-expressing, normal epithelial cells is not likely to occur since all normal cells in these compartments, including mesothelial cells, are AMOC-31-negative. The main conclusions from this study are as follows. First, the treatment can be given safely with only minor toxicity. Secondly, an antitumour response can occur upon treatment with redirected autologous T lymphocytes and, thirdly, this antitumour response is accompanied by a strong inflammatory reaction. Antitumour activity is demonstrated immunocytologically by visualisation of the rapid formation of lymphocyte-tumour conjugates (Fig. 1), which is followed by a disappearance or strong reduction of the number of tumour cells (Table 2). These results are corroborated by the observation that the concentrations of CEA in the ascites fluid of patient 9 decreased significantly after therapy (Fig. 2). As the volume of ascites in patient 9 was found to decrease substantially following treatment, this decreased concentration of CEA may indeed be due to local tumour reduction. Another tumour marker, TAG72.4, especially related to colon and gastric carcinoma, also decreased over this period (data not shown). Treatment in the absence of bsmAb, resulted in much less pronounced conjugate formation between the activated T lymphocytes and the tumour cells, although conjugates were observed to a limited extent. This conjugate formation occurring between the activated T cells and target cells might be considered as a nonspecific interaction event preceding specific MHC-TcR recognition, and has been described extensively by others [28, 30].

Granulocyte influxes as well as increased levels of TNF, IL-6 and CRP following treatment with bsmAb-redirected activated T lymphocytes were observed locally and suggest an inflammatory reaction occurring as a result of the presence of bsmAb since these phenomena were seen only to a limited extent in the absence of bsmAb. Most likely the inflammatory cascade is started by crosslinking of (helper) T lymphocytes with the tumour cells. The T lymphocytes thereby become triggered to produce cytokines such as IFNγ and TNF. The latter could, in turn, induce macrophages and mesothelial cells to produce IL-6 and possibly IL-8, which would further mediate the inflammatory cascade as reflected in the observed granulocyte influx. Release of cytokines may also explain the observed ICAM-1 up-regulation in the tumour cells. ICAM-1/LFA-1

interaction has been shown to be an important event in both tumour cell killing and tumour infiltration by cytotoxic effector cells [1, 4, 14, 15, 23, 29, 37]. Although no data are available for this, the granulocytes observed after treatment might contribute to the observed rapid clearance of tumour cells. IL-6 was found to be increased both intraperitoneally and, to a much lower extent, systemically. Since the peak concentration of serum IL-6 was much lower than the local concentration, the IL-6 in blood most probably results from leakage of intraperitoneally produced IL-6. Systemic IL-6 induces CRP production as shown in Fig. 6 and might be responsible for the observed mild (below 39°C) fever. bsmAb-induced production of cytokines by activated T lymphocytes has been reported and might have an important antitumour effect, since it has been shown that not only direct T-cell-mediated tumour cell killing but also cytokine-mediated tumour growth inhibition takes place [27, 35]. Direct evidence for bsmAb-mediated T-cell activation in vivo after the first few days of treatment is provided by the fact that increased local amounts of sCD8 could be measured (Fig. 9). Elevated levels of sCD8 have been shown to correlate with T cell activation [38]. The study described here has shown that both a local antitumour response and a local inflammatory reaction during treatment with bsmAb-targeted T lymphocytes occurs in intraperitoneally and intrapleurally treated carcinoma patients. The patients presented in this study also suffered from a large tumour burden in the areas mentioned, and it is an important finding that significant local reduction of such a tumour load can be accomplished by the treatment described here. Further development of this protocol may prove promising in the local treatment of minimal residual disease, for example after chemotherapy.

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