Int J Colorectal Dis (2000) 15:197–205
DOI 10.1007/s003840000243

ORIGINAL ARTICLE

Angela Märten
Carsten Ziske
Björn Schöttker
Sabine Renoth
Silvia Weineck
Peter Buttgereit
Frank Schakowski
Dietrich Klingmüller
Christian Scheffold
Alexander von Rücker
Tilman Sauerbruch
Ingo G.H. Schmidt-Wolf

Increase in the immunostimulatory effect of dendritic cells by pulsing with serum derived from pancreatic and colorectal cancer patients

Accepted: 22 June 2000
Published online: 1 August 2000

A. Märten · C. Ziske · B. Schöttker
S. Renoth · S. Weineck · P. Buttgereit
F. Schakowski · T. Sauerbruch
I.G.H. Schmidt-Wolf (✉)
Medizinische Klinik und Poliklinik I,
Rheinische Friedrich-Wilhelms-Universität,
Sigmund-Freud-Strasse 25,
53105 Bonn, Germany
e-mail: picasso@uni-bonn.de
Tel.: +49-228-2875507
Fax: +49-228-2875849

D. Klingmüller · A. von Rücker
Institut für Klinische Biochemie,
Rheinische Friedrich-Wilhelms-Universität, 53105 Bonn, Germany

C. Scheffold
Bone Marrow Transplantation Program,
Stanford University Medical Center,
Stanford, Calif., USA

Abstract Both we and others have observed a relative resistance of solid tumor cells to immunological effector cells in vitro, which may be one reason for the clinical phenomenon of resistance of patients with pancreatic carcinoma or other solid tumors to immunological therapeutic approaches. Dendritic cells (DC) are professional antigen-presenting cells which can process and present tumor-associated antigens such as CA 19-9. Here we tested DC pulsed with serum containing CA 19-9 for their capacity to stimulate immunological effector cells against pancreatic carcinoma cells. Coculture of immunological effector cells with DC led to a significant increase in cytotoxic activity as measured by a lactic dehydrogenase release assay. Most interestingly, cytotoxic activity against tumor cells was further increased using DC pulsed with patient-derived CA 19-9 containing serum. Similar results have been obtained using either autologous or allogeneic serum from patients with pancreas carcinoma. The effect of serum on the cytotoxicity of effector cells increased in a dose-dependent manner. Interestingly, heat inactivation led to a significant loss of immunostimulatory capacity of the serum. Cytotoxicity was partially inhibited by using an antibody directed against CA 19-9 on the surface of the target cells. Best results were obtained when adding CA 19-9 protein to CA 19-9 containing serum for pulsing of DC. In conclusion, DC pulsed with CA 19-9 containing serum increased the cytotoxic activity of immunological effector cells against pancreatic cancer cells. DC pulsed with CA 19-9 containing serum with or without additional exogenous CA 19-9 protein may have an impact on immunotherapeutic protocols for patients with CA 19-9 secreting tumors.

Keywords Dendritic cells · Immunological effector cells · Immunotherapy · CA 19-9

Introduction

Both we and others have observed a relative resistance of pancreatic and colon carcinoma cells to immunological effector cells in vitro [1]. This resistance may be one reason for the clinical phenomenon of these tumors withstanding immunotherapeutic approaches in humans. Dendritic cells (DC) may be used to overcome such a resistance since DC play a major role in the immune response to tumor-associated antigens (TAA) in humans. Peripheral DC capture and process antigens, express lymphocyte costimulatory molecules, migrate to lymphoid organs, and secrete cytokines to initiate immune responses [2]. DC can now be readily obtained in sufficient quantities to allow biological studies and clinical trials. Therefore DC represent a powerful tool for manipulating the immune attack against TAA. Only few TAA have been characterized, one of which, CA 19-9, a carbohydrate-derived marker related to the Lewis blood group antigen, is elevated in more than 70% of patients

with pancreatic cancer. Pulsing of DC with exogenous CA 19-9 protein increases their immunostimulatory capacity against CA 19-9 positive tumor cells [3].

Here, we tested dendritic cells pulsed with serum derived from patients with elevated CA 19-9 protein levels for their immunostimulatory capacity of immunological effector cells against colon and pancreatic carcinoma cells.

Materials and methods

Generation of dendritic cells

Peripheral blood lymphocytes were isolated from buffy coats or blood from patients with pancreatic carcinoma by Ficoll density gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway). Blood was drawn according to our protocol, accepted by the local ethics committee. The cells were allowed to adhere in six-well plates in a density of 5×10^6 cells/ml for 1 h at 37°C in RPMI 1640 with 10% autologous serum. The nonadherent cells were collected for generating immunological effector cells (see below). The adherent cells were cultured in 2 ml RPMI 1640 with autologous serum, 750 U/ml granulocyte-macrophage colony stimulating factor (GM-CSF) and 500 U/ml interleukin (IL) 4 (Essex Pharma, Nuremberg Germany) per well.

Generation of immunological effector cells

Effector cells were generated as described previously [4, 5]. In brief, nonadherent Ficoll separated human peripheral blood mononuclear cells were prepared and grown in RPMI 1640 medium (Gibco BRL, Berlin, Germany), consisting of 10% fetal calf serum (Gibco BRL), 25 mM hydroxyethylpiperazine ethane sulfonic acid, 100 U/ml penicillin, and 100 U/ml streptomycin. Human recombinant interferon-γ (1000 U/ml; Boehringer-Mannheim, Mannheim, Germany) was added on day 0. After 24 h of incubation 50 ng/ml of an antibody against CD3 (Orthoclone OKT3, Cilag, Sulzbach, Germany), 100 U/ml IL-1β, and 300 U/ml IL-2 (Boehringer-Mannheim) were added. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2, pH 7.4 and subcultured every 3 days in fresh medium and IL-2 at 3×10^6 cells/ml.

Pulsing of dendritic cells with exogenous CA 19-9

Dendritic cells were pulsed on day 1 with 100 U/ml CA 19-9 protein (Calbiochem, Bad Soden, Germany) or 100 ng myoglobin (Sigma, Deisenhofen, Germany) as irrelevant protein. Pulsing was stopped on day 4 of culture by change of medium.

Pulsing of dendritic cells with serum

The dendritic cells were pulsed on day 1 with autologous or allogeneic serum from patients with pancreas carcinoma or from healthy donors in concentrations varying from 10% to 50% of medium. The serum was either heat-inactivated for 30 min at 56°C or native. Pulsing was stopped on day 4 by change of medium.

Origin of serum

Serum used for pulsing of DC was derived from patients with pancreatic or colorectal cancer. Serum was obtained from supernatant after Ficoll density gradient. CA 19-9 levels were determined by electro-chemiluminescence immunoassay (ELECSYS; Boehringer-Mannheim). The interassay coefficient of variation (n=10) was 3.6%, and the intra-assay coefficient (n=10) was 1.6%.

Coculturing of effector cells with autologous DCs

Effector cells were harvested on day 7 and cocultured for 8 days with dendritic cells at a stimulator to responder ratio of 1:5.

Cytotoxicity assay

A CytoTox 96 nonradioactive assay (Promega, Madison, Wis., USA) was used to measure cytotoxic activity, which is a colorimetric alternative to the ^{51}Cr release assay. This quantitatively measures lactate dehydrogenase (LDH) released upon cell lysis in the same way as ^{51}Cr-labeled LDH in culture supernatants was measured after 30 min of incubation using a coupled enzymatic assay. The density of color formed is proportional to the number of lysed cells. Absorbance data were collected using a 96-well plate reader at 490 nm. Target cells (20,000) were plated in triplicate in a U-bottom 96-well tissue culture plate and incubated for 4 h with various ratios of effector to target cells. After incubation 50-µl aliquots from each well were transferred to a fresh 96-well plate. The quantity of 50 µl of the substrate mix was added to each well and incubated at room temperature for 30 min in the dark. Before measuring 50 µl of a stop solution was added to each well. Maximal release of LDH was performed by incubating the target cells with 0.1% Igepal (anionic detergents from Sigma, Deisenhofen Germany). Target cells without effector cells were used as negative control (spontaneous release). The calculation of cytotoxicity was made by the following formula: percentage cytotoxicity=[(experimental absorbance minus spontaneous release of effector cells) minus spontaneous release of target cells])/(maximal release minus spontaneous release of target cells).

Flow cytometry

Cells were incubated with the corresponding antibodies on ice for 15 min. Cells were washed with phosphate-buffered solution with (PAA, Cölbe, Germany) with 1% for bovine serum albumin from (Sigma). Dual-color flow cytometric analysis was performed on a Coulter Epics XL Cytometer (Coulter Immunotech, Krefeld, Germany). Data from 30,000 cells were collected and analyzed. DC were phenotyped with the following monoclonal markers: CD1a, CD80, CD86, HLA-ABC, HLA-DR (all from Pharmingen, Hamburg, Germany), CD83 (Coulter Immunotech), and CMRF-44 (a kind gift from D.N.J. Hart, New Zealand). CA 19-9 staining of target cell lines was performed with a monoclonal antibody obtained from Coulter Immunotech.

Cell lines

DAN-G cells (pancreas carcinoma) were purchased from Deutsche Sammlung für Mikroorganismen und Zellkultur (Braunschweig, Germany). Colo 205 (colon carcinoma). Cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum (PAA), 100 U/ml penicillin, and 100 U/ml streptomycin (Seromed, Jülich, Germany). The cell lines expresses CA 19-9 on their surface.

Primary cultures from colorectal carcinoma

Primary cultures were established by a single-cell isolation method from patients' colorectal tumors as described elsewhere [6] after obtaining informed consent according to the policy of our institution. In brief, adjunct non-carcinoma-containing tissue was re-

moved by scalpel, and tumors were subsequently cut into fragments as small as possible and then forced through a nylon mesh (pore size 100 µm). Tumor fragments were then put into isolation solution [Hank's balanced salt solution (Gibco) containing 100 U/ml DNase I (Boehringer); 50 U/ml collagenase III, 150 U/ml collagenase IV (Biochrom); 200 U/ml hyaluronidase (Sigma); and 0.08 U/ml insulin (Hoechst)} at 37°C on a shaker for 15 min. Supernatant was removed and centrifuged for 5 min at 400 *g*. The pellet was resuspended in 10 ml erythrocyte lysation buffer (8.29 g/l NH_4Cl, 1.0 g/l $KHCO_3$, 0.0371 g/l EDTA in Aqua, all from Sigma) and incubated at room temperature for 15 min. Cells were centrifuged (7 min at 400 *g*) and the pellet was resuspended at a density of 10^6–10^7 cells/ml in Leibovitz medium (containing 10% fetal calf serum, 1 mM L-glutamine, 1×MEM vitamins, 2.5 mg/ml transferrin, 1 g/l sodium bicarbonate, 1 g/l glucose, 80 U insulin, 10 mg/ml gentamicin, all from Gibco). The isolation and erythrocyte-depletion steps were performed three times. Cells were spliced 1:2 when confluent. The cells were adherent and showed identical morphology typical of colorectal cells. Cells were epithelial-like; they showed no fibroblast morphology, i.e., they were not bipolar spindle-shaped. Cultures contaminated with fibroblasts were rejected. Primary cultures were used in a cytotoxicity assay after a maximum of two passages.

Blockage of stimulatory effect of pulsing by addition of an anti-CA 19-9 antibody

A total of 2×10^6 target cells (DAN-G) were preincubated with 6.25 µg monoclonal antibody against CA 19-9 for 15 min before addition of effector cells in a cytotoxicity assay.

Results

In vitro generation of DC

DC were generated from buffy coats or blood from patients with colorectal or pancreatic carcinoma using GM-CSF and IL-4 as described above. Adherent cells showed cytoplasm processes typical of DC. After coculturing with effector cells DC formed typical clusters. The percentage of monocytes ($CD14^+$) declined during culture; on day 14 the purity of DC was 79.1±0.5% as determined by HLA-DR positivity and CD14 negativity in flow cytometric analysis.

Cell surface marker expression of DC cultures

DC were stained with various monoclonal antibodies to determine the expression of cell surface markers. DC cultures displayed CMRF-44 and CD83, two markers known to be expressed on DC (Table 1).

Effect of pulsing of DC with patient-derived CA 19-9 containing non-heat-inactivated serum

In further experiments DC derived from healthy individuals were pulsed with serum derived from patients with metastatic pancreatic cancer with elevated CA 19-9 se-

Table 1 Immunophenotype of dendritic cell culture (percentages). Flow cytometric analysis of cell surface expression of DC cultures. Peripheral blood mononuclear cells were cultured with IL-4 and GM-CSF for up to 13 days as described in the text. Cells were either unstimulated or stimulated with human albumin and cocultured with effector cells. After incubation, cells were stained with various antibodies and analyzed by flow cytometry. Data are shown as mean ±standard error from four separate experiments

	Day of culture (coculture with effector cells at day 7)					
	1	3	5	9	11	13
CD1a expression						
On native DC	0.4±0.2	11.1±1.7	13.5±1.2	19.2±3.8	34.8±1.3	*25.7*±1.6
On stimulated and cocultured DC	–	–	–	30.0±1.5	49.4±3.3	38.8±1.8
CD80 expression						
On native DC	10.7±1.6	20.5±0.8	15.6±3.1	28.3±0.2	50.3±0.8	47.2±4.1
On stimulated and cocultured DC	–	–	–	45.5±0.7	54.8±3.1	56.8±1.3
CD83 expression						
On native DC	8.2±1.0	21.0±1.9	11.2±1.1	24.4±0.9	35.8±5.8	35.0±7.0
On stimulated and cocultured DC	–	–	–	37.1±0.7	37.9±5.0	42.5±0.9
CD86 expression						
On native DC	35.9±2.3	28.3±2.1	32.2±2.8	42.8±7.9	57.1±4.3	52.1±4.0
On stimulated and cocultured DC	–	–	–	60.8±1.8	65.8±1.8	65.3±0.2
HLA-ABC expression						
On native DC	70.7±5.5	75.2±0.2	65.6±10.3	86.5±1.8	87.9±1.8	85.4±2.9
On stimulated and cocultured DC	–	–	–	90.9±0.9	93.8±0.6	92.9±0.2
HLA-DR expression						
On native DC	27.9±0.3	25.3±0.4	25.5±1.0	51.2±3.1	47.9±1.5	50.9±2.9
On stimulated and cocultured DC	–	–	–	75.7±5.4	85.9±6.1	79.1±0.5
CMRF-44 expression						
On native DC	12.0±1.5	28.7±3.3	24.1±3.2	52.5±2.0	49.7±4.1	54.5±3.0
On stimulated and cocultured DC	–	–	–	60.8±1.8	51.2±4.5	55.2±1.8

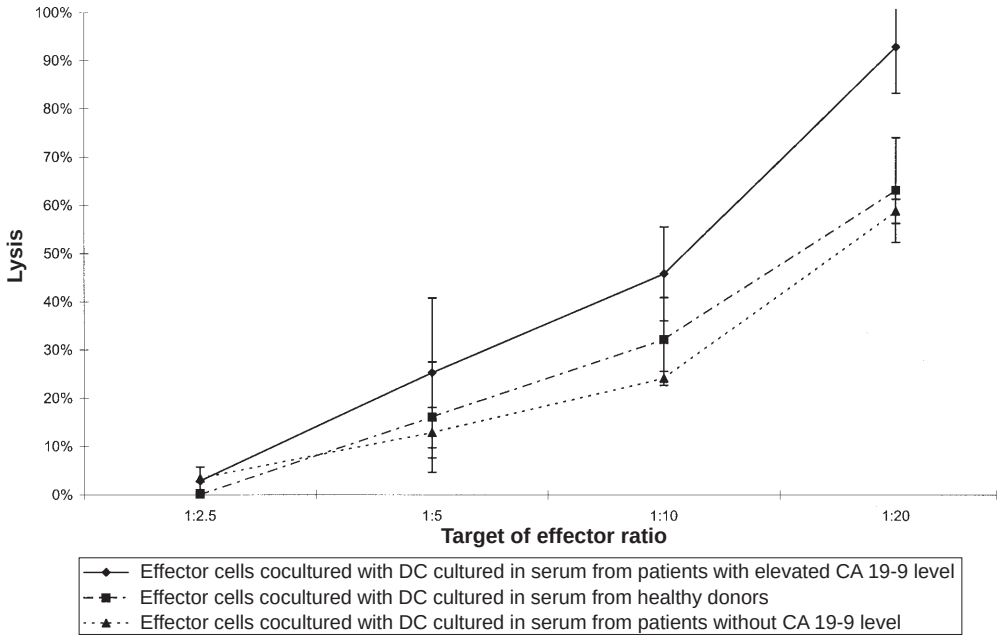

Fig. 1 Pulsing of dendritic cells with CA 19-9 containing patient-derived non-heat-inactivated serum. DC derived from healthy individuals were generated and tested for their capacity to stimulate immunological effector cells. Autologous effector cells were cocultured from days 7 to 14 with DC as described in the text. DC were pulsed either with non-heat-inactivated CA 19-9 positive serum from patients with pancreatic cancer, serum from healthy donors, or serum from patients with pancreatic cancer without elevated CA 19-9 serum levels. The serum levels from the four different donors with elevated serum levels varied from 5000 to 8000 U/ml; the serum levels from the two patients without elevated serum levels were below 50 U/ml. Cytotoxic activity of effector cells at various effector to target cell ratios was measured by LDH release assay. DAN-G cells were used as targets. Results represent data from four separate experiments. Data are shown as mean ±standard error

Fig. 2 Titration of non-heat-inactivated serum for pulsing of DC cocultured effector cells. Effector cells were cocultured from days 7 to 14 with autologous DC cultures 7 days of age as described in the text. DC pulsed with various concentrations of non-heat-activated serum. Serum containing various amounts of CA 19-9 was derived from various patients. Cytotoxic activity of effector cells at various effector to target cell ratios was measured by LDH release assay. DAN-G cells were used as targets. Data from three experiments are shown as mean ±standard error

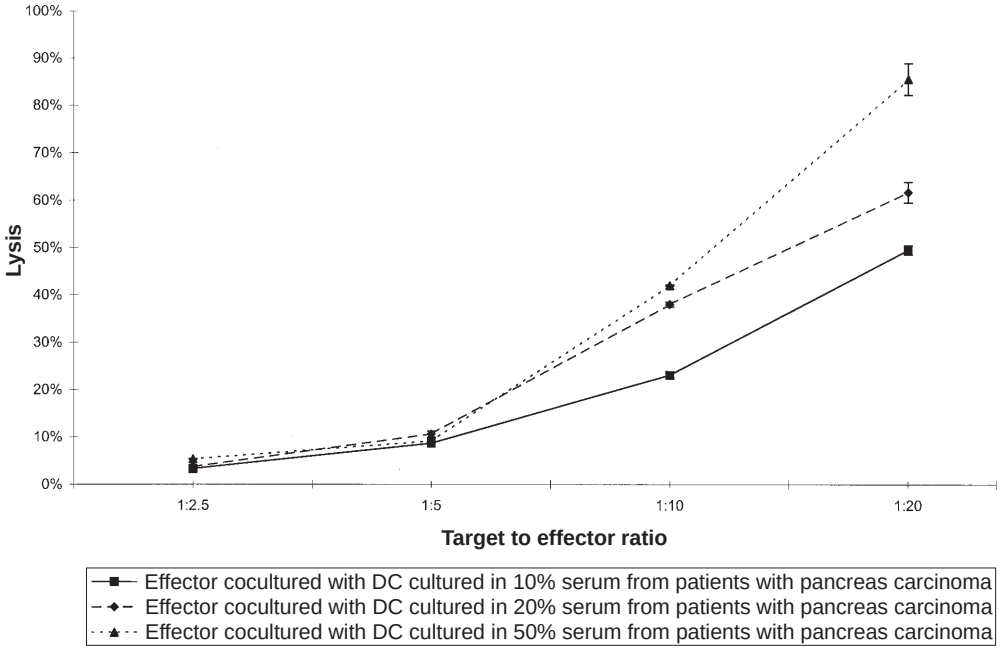

Table 2 Titration of non-heat-inactivated serum for pulsing of DC cocultured effector cells (percentages). Effector cells were cocultured from days 7 to 14 with autologous DC from healthy donors as described in the text. DC were pulsed with various amounts of non-heat-activated serum and were cocultured with effector cells. Serum from three patients was titrated to equivalent doses of CA 19-9 (100 U/ml) and the results were compared with the immunostimulatory capacity of exogenous CA 19-9. Cytotoxic activity of effector cells at various effector to target cell ratios was measured by LDH release assay. DAN-G cells were used as targets

	Effector to target ratio				Serum concentration in medium
	2.5:1	5:1	10:1	20:1	
DC pulsed with patient serum containing 100 U/ml CA 19-9					
From patient 1	6.8	17.5	42.2	80.7	5.3
From patient 2	11.3	20.8	46.9	83.9	18.9
From patient 3	6.5	18.4	37.2	78.4	29.6
Mean ±standard error	8.2±2.2	18.9±1.4	42.1±3.9	81.0±2.3	
DC pulsed with 100 U/ml exogenous CA 19-9 (n=3)	–	21.8	58.3	86.0	10 Serum from a healthy donor

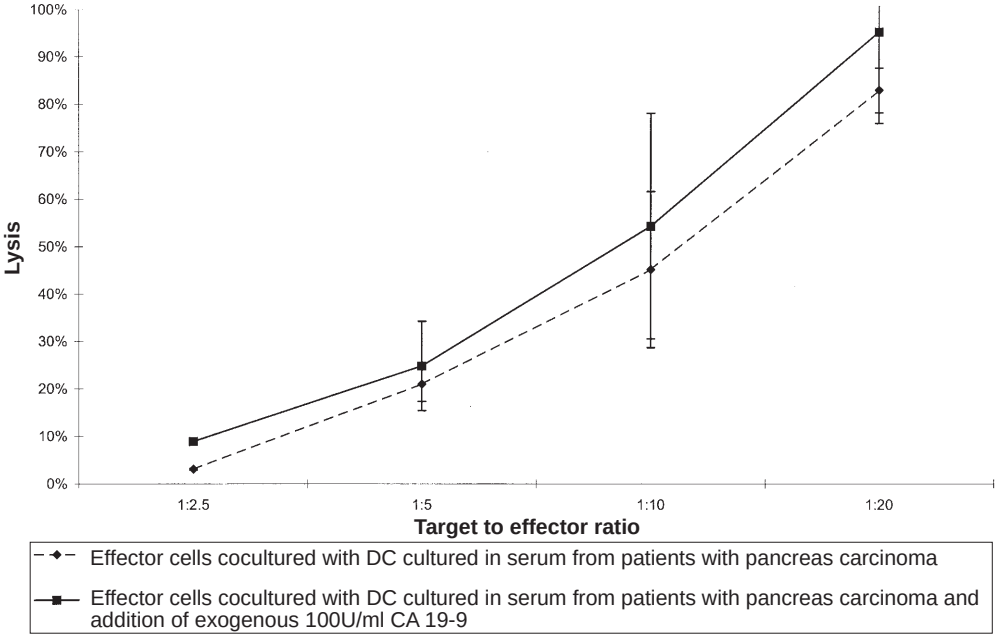

Fig. 3 Pulsing with non-heat-inactivated serum with additional CA 19-9. DC were generated from healthy individuals were cultured with IL-4 and GM-CSF for up to 14 days as described in the text. Cells were either unstimulated or were stimulated with human albumin; unstimulated and stimulated dendritic cells were cocultured with effector cells on day 7. Serum was derived from patients with pancreatic carcinoma; the serum level varied between 1000 and 5000 U/ml. In addition, exogenous CA 19-9 was added. Data are shown as mean ±standard error from three separate experiments

rum levels between 5000 and 8000 U/ml. DC were tested for their capacity to stimulate effector cells. Pulsing of DC with non-heat-inactivated serum derived from patients with pancreas carcinoma led to significantly greater cytotoxic activity than that of DC cultured in non-heat-inactivated serum from healthy donors. For example, lysis increased from 63.2±10.8% to 92.8±9.6% at an effector to target cell ratio of 20:1 (Fig. 1; P=0.05).

The increase in cytotoxic activity mediated by non-heat-inactivated patient-derived serum was dose dependent. This was determined by pulsing DC with various concentrations of serum (Fig. 2), containing 5000 U/ml CA 19-9. Furthermore, serum derived from various patients with various CA 19-9 serum levels showed similar effects on cytotoxic activity when titrated to a constant dose of 100 U/ml CA 19-9 (Table 2). This effect was independent of the serum concentration in medium. Serum derived from healthy individuals did not increase the stimulatory activity of DC. The cytotoxicity was seen independent of the allogeneic or autologous character of the serum (data not shown).

The increase in cytotoxic activity mediated by pulsed DC was further augmented by addition of exogenous CA 19-9. For example, lysis increased from 82.9±19.3% to 95.8±5.1% at a target to effector cell ratio of 1:20 (Fig. 3; P=0.03). Concentrations higher than 500 U/ml exogenous CA 19-9 lead to an inhibition of tumor cell lysis (Fig. 4).

The increase in the stimulatory effect of DC by non-heat-inactivated serum was demonstrated using autologous DC, autologous serum, and the pancreas carcinoma cell line DAN-G. Lysis increased from 35.0±2.5% to

77.7±8.9% at an effector to target cell ratio of 40:1 (Fig. 5; *P*=0.04).

Cytotoxicity of cocultured immunological effector cells against autologous primary cultures

When using a complete autologous model with primary cultures of colorectal carcinoma cells as targets, effector cells also showed an increase in target cell lysis. Effector cells cocultured with autologous DC pulsed with autologous serum and exogenous CA 19-9 showed an increase in lysis from 40.6±11.5% to 98.3±1.7% as compared to addition of exogenous CA 19-9 to DC cultured in heat-inactivated serum at an effector to target cell ratio of 80:1 (Fig. 6).

Partial blockage of stimulating effect of pulsing by addition of an anti-CA 19-9 antibody

Addition of a monoclonal antibody against CA 19-9 to CA 19-9 positive target cells (DAN-G cells) decreased the cytotoxicity of effector cells cocultured with dendritic cells, which were pulsed either with exogenous CA 19-9 or non-heat-inactivated serum from patients with elevated CA 19-9 levels. For example, lysis decreased from 49.1±2.8% after stimulation with 250 U/ml CA 19-9 to 13.8±3.7% at an effector to target cell ratio of 20:1 (Fig. 7; *P*=0.01).

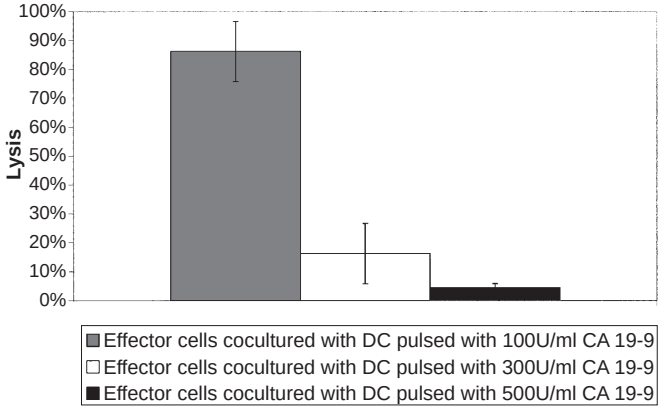

Fig. 4 Titration of exogenous CA 19-9 for pulsing of DC cocultured effector cells. Effector cells were cocultured from days 7 to 14 with autologous DC cultures 7 days of age as described in the text. DC were pulsed with various concentrations of exogenous CA 19-9. Cytotoxic activity of effector cells at an effector to target cell ratio of 40:1 was measured by LDH release assay. DAN-G cells were used as targets. Data from three experiments are shown as mean ±standard error

Discussion

The ability of malignant cells to survive exposure to cytotoxic agents is a major obstacle to an effective therapy in cancer patients. Immunological effector cells such as lymphokine-activated killer cells [7], tumor-infiltrating lymphocytes [8] or natural killer-like T cells termed cytokine-induced killer cells [4] may be suitable to remove residual tumor cells resistant to chemotherapy. Large

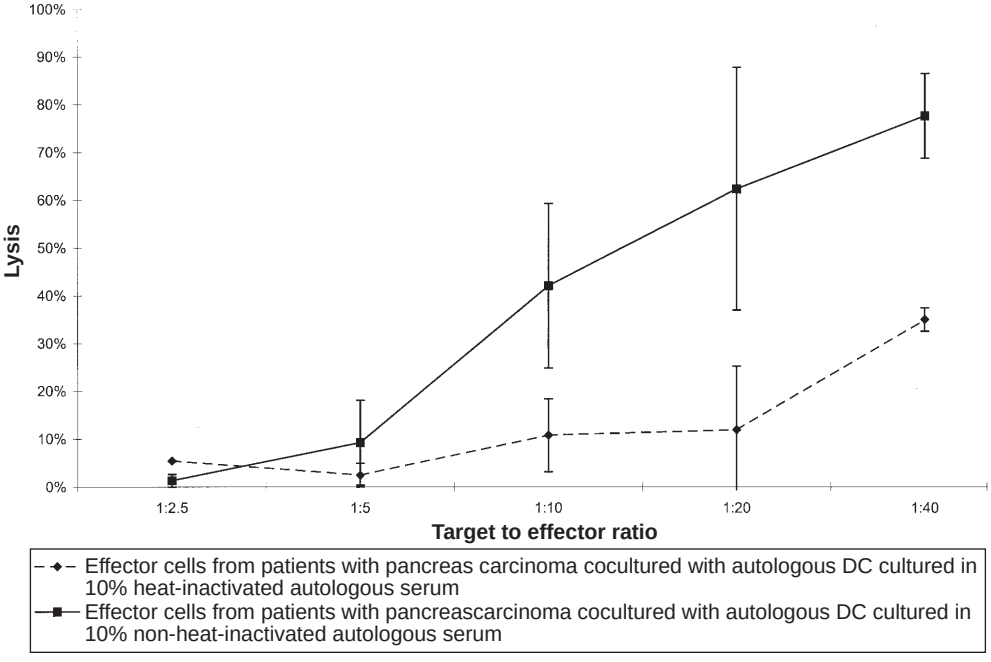

Fig. 5 Cytotoxicity against allogeneic tumor cells after pulsing with autologous non-heat-inactivated serum. DC derived from patients with metastatic pancreatic cancer with elevated CA 19-9 serum levels were generated and tested for their capacity to stimulate effector cells. Effector cells were cocultured from days 7 to 14 with autologous DC. DC were pulsed with non-heat-inactivated serum. Cytotoxic activity of cocultured effector cells at various effector to target cell ratios was measured by LDH release assay. DAN-G cells were used as targets. Results represent data from five separate experiments and are shown as mean ±standard error

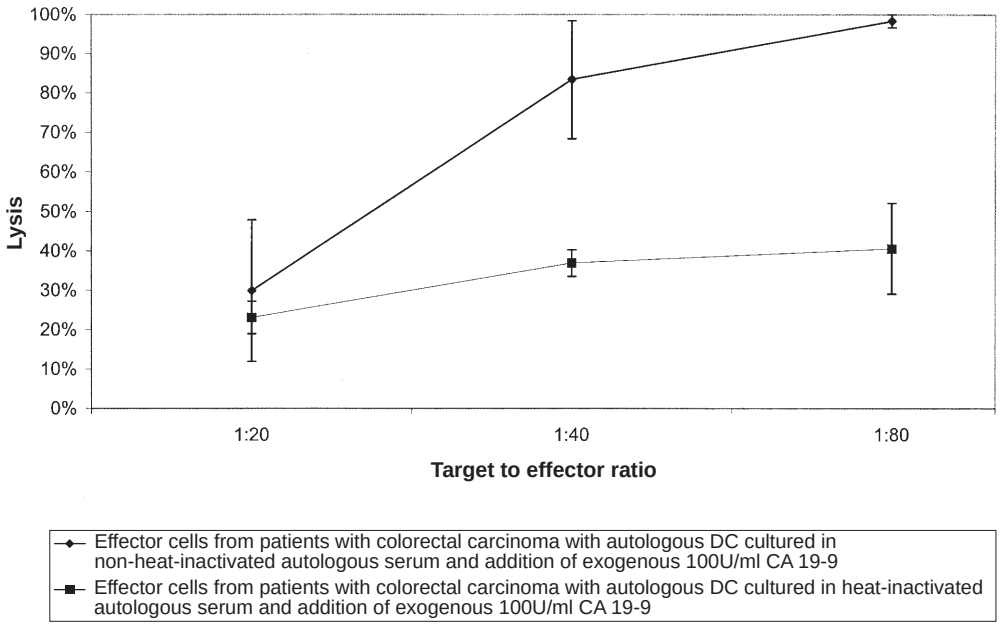

Fig. 6 Cytotoxicity against autologous tumor cells after pulsing with autologous non-heat-inactivated serum. DC derived from patients with metastatic colorectal cancer were generated and tested for their capacity to stimulate effector cells. Effector cells were cocultured from days 7 to 14 with autologous DC. DC were pulsed with non-heat-inactivated serum. In addition, exogenous CA 19-9 was added. Cytotoxic activity of effector cells at various effector to target cell ratios was measured by a LDH release assay. Autologous tumor cells were isolated as described in the text and were used as targets. Data from two experiments are shown as mean ±standard error

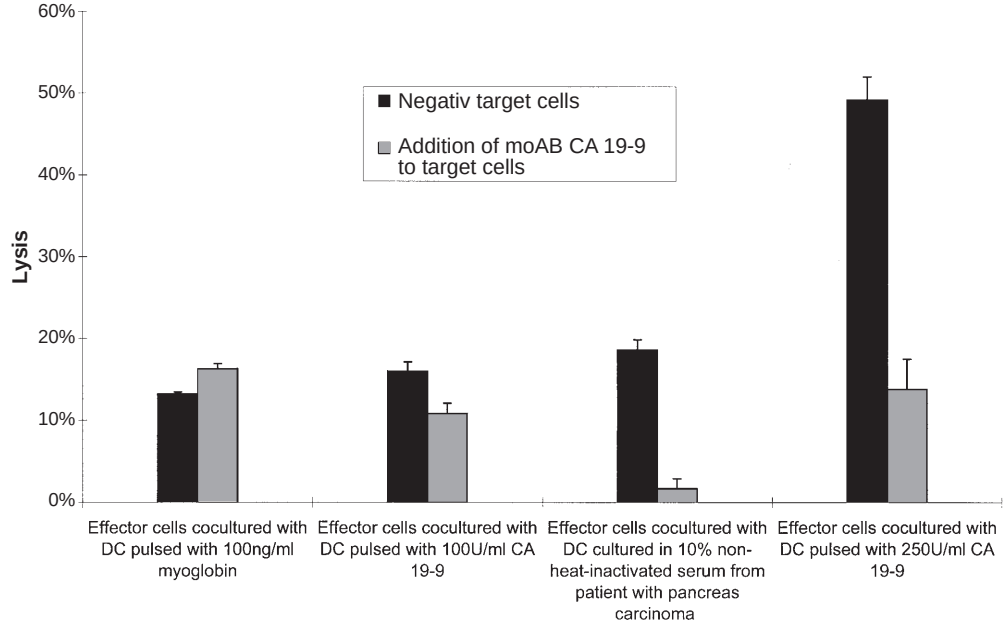

Fig. 7 Partial blockage of stimulating effect of pulsing by addition of an anti-CA 19-9 antibody. DC were either unstimulated or stimulated with myoglobin, with serum from patients with elevated CA 19-9 levels or with exogenous CA 19-9. Cytotoxic activity of effector cells was measured by LDH release assay at an effector to target cell ratio of 20:1. DAN-G cells were used as targets. For blockage of CA 19-9 on target cells, antibody against CA 19-9 was added before LDH assay was performed. Data are shown as mean ±standard error from three separate experiments

numbers of these cells are necessary for effective immunotherapy. However, in some patients it is difficult to obtain sufficient effector cell numbers since these cells grow poorly in vivo.

We reported a protocol generating large numbers of efficient immunological cells [5, 9]. Immunological effector cells were generated by incubation of peripheral blood lymphocytes with anti-CD3 monoclonal antibody, IL-2, IL-1, and interferon-γ [10]. These effector cells possess greater cytotoxicity and a higher proliferation rate than lymphokine-activated killer cells [5, 11, 4]. They are derived from T cells [12] and are capable of promptly producing several cytokines including IL-4 and interferon-γ [13].

Pancreatic and colon cancer cells have been shown to be more resistant to immunological effector cells than lymphoma cells. A possible reason for this relative resistance is the lack of CD80 expression on pancreatic and colon cancer cells [14]. The absence of costimulatory molecules has been described as one of the reasons for the resistance of these tumor entities to immunological effector cells [14] which kill their targets depending on

the CD80 and CD86 expression on the target cell surface. This resistance can be overcome by coculture of immunological effector cells with DC. DC cultures can be grown both from healthy individuals and from cancer patients [14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. In our experiments almost 60% of DC cultures expressed CMRF-44, a DC-specific cell surface marker, after 13 days of cultivation and coculturing with effector cells. Similarly, CD80 and CD86 expression increased to 65%. As outlined above, the expression of these costimulatory molecules is known to be essential for the immunostimulatory effect of DC.

We have recently shown that DC pulsed with CA 19-9 protein increases the cytotoxic effect of effector lymphocytes on Colo 205 and DAN-G cells significantly [3]. Optimal cytotoxic activity was reached by prior incubation of DC with CA 19-9 protein. These results are in accordance with the results obtained with coculture of DC with carcinoembryonic antigen peptide [27].

We tested the antigenic potential of serum derived from patients with elevated CA 19-9 levels. We demonstrated that a dose-dependent immunostimulatory effect is obtained with such a serum for pulsing of DC, comparable to that of exogenous CA 19-9. However, addition of CA 19-9 containing serum and exogenous CA 19-9 produced the best results. We consider this effect to be partially CA 19-9 dependent, because serum derived from healthy individuals had no effects on immunological cells, and antibody against CA 19-9 reduced the cytotoxicity of cocultured lymphocytes. Presumably other proteins in addition to CA 19-9 may also be responsible for the immunomodulatory capacity of patient-derived serum.

Either autologous or allogeneic CA 19-9 containing serum can be used for immunostimulation. However, it appears important that the serum is not heat-inactivated. Heat inactivation leads to a significant loss of immunostimulatory capacity of the serum.

In conclusion, DC increased the cytotoxic activity of immunological effector cells against colon carcinoma and pancreatic cancer cells. The cytotoxic activity was further increased by pulsing DC with serum derived from patients with elevated CA 19-9 serum levels, in particular when exogenous CA 19-9 was added. Similar results were obtained with immunological cells derived from patients with pancreatic carcinoma. Furthermore, we demonstrated cytotoxicity of immunological effector cells from patients with colorectal carcinoma against autologous primary cultures expressing CA 19-9. DC pulsed with CA 19-9 containing serum with or without additional exogenous CA 19-9 protein may have an impact on immunotherapeutic protocols for patients with CA 19-9 secreting tumors.

Acknowledgements We thank BONFOR for a generous grant, Essex, Munich, for their kind gift of IL-4 and D.N.J. Hart, Christchurch, New Zealand, for his kind gift of the CMRF-44 antibody.

References

1. Bean P, Mazumder A (1992) Resistance of different tumor cells to lysis by lymphokine activated killer cells can be mediated by distinct mechanisms. Immunobiology 185:63–77
2. Banchereau J, Steinman R (1998) Dendritic cells and the control of immunity. Nature 392:245–252
3. Märten A, Lefterova P, Weineck S, Buttgereit P, Huhn D, Sauerbruch T, Schmidt-Wolf I (2000) Increase of the immunostimulatory effect of dendritic cells by pulsing with CA 19-9-protein. J Immunther (in press)
4. Schmidt-Wolf I, Lefterova P, Johnston V, Neubauer A, Huhn D, Negrin R, Blume K (1994) Propagation of large numbers of T cells with natural killer cell markers. Br J Haematol 87:453–458
5. Schmidt-Wolf I, Negrin R, Kiem H, Blume K, Weissmann I (1991) Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. J Exp Med 174:139–149
6. Kemmner W, Schlag P, Brossmer R (1987) A rapid and simple procedure for dissociation of tumor tissue from the human colon. J Cancer Res Clin Oncol 113:400–401
7. Grimm E, Mazumder A, Zhang H, Rosenberg S (1982) Lymphokine activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by IL-2 activated autologous human peripheral blood lymphocytes. J Exp Med 155:1823–1841
8. Rosenberg S, Spiess P, Lafreniere R (1986) A new approach to the adoptive immunotherapy of cancer with tumor infiltrating lymphocytes. Science 233:1318–1321
9. Schmidt-Wolf I, Finke S, Trojaneck B, Denkena A, Lefterova P, Schwella N, Heuft H, Prange G, Korte M, Takeya M, Dorbic T, Neubauer A, Wittig B, Huhn D (1999) Phase I clinical study applying autologous immunologic effector cells transfected with the interleukin-2 gene in patients with metastatic renal cancer, colorectal cancer and lymphoma. Br J Cancer 81:1009–1061
10. Mehta B, Schmidt-Wolf I, Weissmann I, Negrin R (1995) Two pathways of exocytosis of cytoplasmic granule contents and target cell killing by cytokine-induced CD3+CD56+ killer cells. Blood 86:3493–3499
11. Margolin K, Negrin R, Wong K, Chatterjee S, Wright C, Forman S (1997) Cellular immunotherapy and autologous transplantation for hematologic malignancy. Immunol Rev 157:231–240
12. Lantz O, Sharara L, Tilloy F, Andersson A, DiSanto J (1997) Lineage relationships and differentiation of natural killer (NK) T cells: intrathymic selection and interleukin (IL)-4 production in the absence of NKR-P1 and Ly49 molecules. J Exp Med 185:1395–1401
13. Leite-DeMoraes M, Moreau G, Arnould A, Machavoine F, Garcia C, Papiernik M, Dy M (1998) IL-4-producing NK T cells are biased towards IFN-gamma production by IL-12. Influence of the microenvironment on the functional capacities of NK T cells. Eur J Immunol 28:1507–1515

14. Huang A, Bruce A, Pardoll D, Levitsky H (1996) Does B7–1 expression confer antigen-presenting cell capacity to tumors in vivo? J Exp Med 183:769–776
15. Hart D (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 90:3245–3287
16. Boczkowski D, Nair S, Snyder D, Gilboa E (1996) Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med 184:465–472
17. Specht J, Wang G, Do M, JS L, Royal R, Reeves M, Rosenberg S, Hwu P (1997) Dendritic cells retrovirally transduced with a model antigen gene are therapeutically effective against established pulmonary metastases. J Exp Med 186:1213–1221
18. Song W, Kong H, Carpenter H, Torii H, Granstein R, Rafii S, Moore M, Crystal R (1997) Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. J Exp Med 186:1247–1256
19. Schuler G, Steinman R (1997) Dendritic cells as adjuvants for immune-mediated resistance to tumors. J Exp Med 186:1183–1187
20. Westermann J, Aicher A, Qin Z, Cayeux S, Daemen K, Blankenstein T, Dörken B, Pezzutto A (1998) Retroviral interleukin-7 gene transfer into human dendritic cells enhances T cell activation. Gene Ther 5:264–271
21. Hsu F, Benike C, Fagnoni F, Lileas T, Czerwinski D, Taidi B, Engleman E, Levy R (1996) Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat Med 2:52–58
22. Nestle F, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D (1998) Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 4:328–332
23. Titzer S, Christensen O, Manzke O, Tesch H, Diehl V, Bohlen H (1997) Vaccination of patients with multiple myeloma using idiotype pulsed autologous dendritic cells derived from highly purified CD34 progenitor cells. Onkologie 20:263
24. Choudhury A, Gajewski J, Liang J, Popat U, Claxton D, Kliche K, Andreeff M, Champlin R (1997) Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 89:1333–1142
25. Westermann J, Aicher A, Qin Z, Daemen K, Mapara M, Döhner H, Blankenstein T, Dörken B, Pezzuto A (1997) Dendritic cells for autologous tumor vaccination in chronic myeloid leukemia. Cancer Gene Ther 4:311
26. Fearnley D, McLellan A, Mannering S, Hock B, Hart D (1997) Isolation of human blood dendritic cells using the CMRF-44 monoclonal antibody: implications for studies on antigen-presenting cell function and immunotherapy. Blood 89:3708–3716
27. Alters S, Gadea J, Sorich M, O'Donoghue G, Talib S, Philip R (1998) Dendritic cells pulsed with CEA peptide induce CEA-specific CTL with restricted TCR repertoire. J Immunother Emphasis Tumor Immunol 21:17–26