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Clinical effects of monoclonal antibody 17-1A combined with granulocyte/macrophage-colony-stimulating factor and interleukin-2 for treatment of patients with advanced colorectal carcinoma

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Abstract Granulocyte/macrophage-colony-stimulating factor (GM-CSF) has previously been indicated to enhance the therapeutic effect of the anti-colorectal carcinoma mAb17-1A as well as to augment in vivo immune effector functions. In vitro interleukin-2 (IL-2) augmented GM-CSF-induced antibody-dependent cellular cytotoxicity, a mechanism considered to be of significance for the therapeutic effect of mAb. A treatment regimen was elaborated that combined mAb17-1A (400 mg at day 3 of a 10-day treatment cycle) with the simultaneous administration of GM-CSF (250 μmg/m^2 once daily) and IL-2 (2.4×10^6 U/m^2 twice daily) for 10 days. The treatment cycle was repeated once a month. Twenty patients with advanced colorectal carcinoma were included in the study. One patient obtained a partial remission and 2 patients stable disease for 7 and 4 months respectively. The median survival time from the start of mAb therapy was 8 months. Owing to allergic reactions, the planned mAb17-1A dose had to be reduced by repeated infusions. At the fourth treatment cycle only 25% received the planned mAb dose. In 3 patients the GM-CSF and IL-2 dose was reduced because of side-effects. The subjective tolerability of the treatment was considered good or acceptable in more than 80% of the patients. The increment in white blood cell subsets induced by the cytokines decreased by increasing number of courses. This particular regimen did not augment the therapeutic effect of mAb17-1A anticipated from in vitro data but rather hampered the clinical effect of the antibody. The reason for this is not clear but a possibility might be the induction of immune suppression in vivo resulting from an impaired human anti-(mouse Ab) and anti-idiotypic antibody response as well as antibody-dependent cellular cytotoxicity, on the basis of a comparison of mAb17-1A/GM-CSF/IL-2- and mAb17-1A/GM-CSF-treated patients.

Key words Colorectal carcinoma · Monoclonal antibodies · GM-CSF-IL-2

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Introduction

Adjuvant treatment of colorectal carcinoma (CRC) patients with the unconjugated mouse monoclonal antibody 17-1A (mAb17-1A) seems promising [23]. In metastatic CRC the clinical effect of mAb17-1A alone is, however, modest, with only occasional responses [22].

There might be several reasons for the failure in metastatic disease. Tumor cells may be resistant to cell death induced by immunotherapeutics. Factors might be produced by the tumor cells impairing immune effector functions of importance for mAb therapy [24]. Such effector mechanisms are antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytolysis and the induction of an idiotypic network response resulting in a humoral and cellular antitumor immunity [5, 9, 10, 32]. To improve the efficacy of mAb therapy in advanced disease, agents such as cytokines might be

added to augment immune functions. Granulocyte/macrophage-colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2) alone or in combination have been shown to enhance ADCC in vitro [11–16, 30]. GM-CSF may augment the induction of an idiotypic network response [6] and has been shown to cooperate with other cytokines to expand specific T cells [25]. IL-2 alone also expands specific T cell populations [7]. Addition of GM-CSF or IL-2 to mAb therapy has been shown to enhance the antitumor response in CRC and malignant melanoma patients [2, 4, 18].

The aim of the present study was to explore the clinical feasibility of combining the murine mAb17-1A with GM-CSF and IL-2 in patients with advanced CRC.

Materials and methods

Twenty patients (12 male and 8 female) with inoperable metastatic CRC entered a phase IB/II trial (Table 1). The median age was 59 years (range 36–73 years). All patients had a Karnofsky index of at least 80%. The tumor cells expressed the tumor-associated antigen CO17-1A/GA73-3, detected by immunohistochemistry [28].

Patients with CNS metastases, HIV, hepatitis, septicemia, symptomatic pulmonary disorders or cardiac disease were excluded. No chemotherapy or immunotherapy was administered for at least 4 weeks before entry to the study. Four patients with rectal carcinoma had received pre-operative irradiation. Six patients had received various chemotherapy regimens 2–14 months prior to the study. The median time from primary surgery to mAb therapy was 20.5 months (range 2–45 months).

Treatment protocol

A 250 $\mu g/m^2$ dose of human recombinant GM-CSF (Leucomax Schering-Plough/Sandoz, Kenilworth, USA; specific activity: 1.2×10^8–5×10^8 U/mg protein) was administered subcutaneously for 10 consecutive days. A dose of 2.4×10^6 U/m^2 recombinant human interleukin-2 (Proleukin, EuroCetus, Amsterdam, The Netherlands; specific activity: 1.8×10^7 U/mg protein) was administered s.c. twice daily for 10 days. At day 3 of a treatment cycle, 400 mg mAb17-1A (mouse IgG2a) (Centocor, Malvern, Pa.) was infused i.v. for 30–60 min. The treatment cycle was repeated every 4th week. Four cycles were given. One patient (patient 3) received two additional treatment cycles.

Before each mAb infusion 1.0 mg mAb17-1A was injected intradermally. The immediate-type skin reactivity (type I) was read after 20 min. This test can reveal low levels of IgE antibodies that in vitro tests may fail to detect [1]. We have previously noted a strong correlation between type I skin reaction and the development of immediate-type allergic reactions (ITAR). Main signs and symptoms of ITAR are bronchospasm, hypotension, fever and chills [8]. Thus, in patients with a positive skin reactivity and/or ITAR against mAb17-1A, the mAb infusion rate was prolonged (4 h) and the dose reduced to 40 mg. When the disease progressed, the patients were offered treatment with 5-FU/α-interferon [20].

Clinical examination and laboratory tests

Before therapy and at the start of each treatment cycle, a physical examination was done. The following laboratory tests were performed: hemoglobin concentration, white blood cell counts with a differential and platelet count once weekly. Liver function tests (aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase, lactate dehydrogenase, and γ-glutamyltransferase) were carried out on days 1 and 14, serum creatinine,

Table 1 Characteristics of the patients at entry into the study. *W* well differentiated, *M* moderately differentiated, *P* poorly differentiated, *IFN* interferon

Patient no.	Sex/age (years)	Primary site of tumor and differentiation	Mod. Astler-Coller class. at surgery	Previous therapy	Time from primary surgery to mAb therapy (months)	Site of metastases
1	M/43	Colon transversum (M)	D	Surgery	3	Liver
2	M/52	Colon ascendens (M)	D	Surgery	2	Peritoneal
3	M/39	Rectum (P)	D	Surgery	2	Peritoneal
4	M/53	Colon ascendens (M)	B1	Surgery	45	Lymph nodes
5	M/48	Rectum (P)	C2	Pre-op. irradiation, surgery	20	Liver
6	F/65	Rectum (P)	C3	Pre-op. irradiation, surgery	33	Local recurrence
7	M/73	Colon ascendens (W)	C3	Surgery	26	Liver, lung
8	F/66	Colon caecum (M)	D	Surgery	18	Peritoneum, ovarium
9	M/64	Colon caecum (M)	D	Surgery		Liver, abdominal wall
10	M/58	Colon sigmoideum (M)	D	Surgery	3	Liver
11	M/70	Rectum (M)	D	Surgery	5	Lung
12	M/60	Rectum (M)	C3	Pre-op. irradiation, surgery, chemotherapy	37	Liver, lung, local recurrence
13	M/70	Colon caecum (M)	C3	Surgery	21	Liver
14	F/42	Colon transversum (M)	D	Surgery, chemotherapy	40	Peritoneum
15	F/61	Colon sigmoideum (M)	B3	Surgery	22	Peritoneum
16	F/36	Colon descenden (M)	D	Surgery	2	Peritoneum
17	F/48	Rectum (M–P)	D	Surgery, irradiation, chemotherapy	22	Pleura, pelvis, thorax skeleton, lymph nodes
18	M/62	Rectum (P)	C2	Pre-op. irradiation, surgery, chemotherapy + IFN	44	Liver, lymph nodes, pancreas
19	F/61	Colon sigmoidum (M)	B2	Surgery, chemotherapy + IFN	28	Peritoneum
20	F/53	Colon ascendens (M)	B2	Surgery, adjuvant chemotherapy	19	Peritoneum

blood urea nitrogen and serum electrolytes at days 1, 5 and 10 of every cycle. The concentration of tumor markers (CEA, CA19-9 and CA-50) in the serum was analyzed at the start of each treatment cycle (CEA-IMX: Abbott, North Chicago, Iu., USA; CA19-9-IMX: Abbott, Delfia; CA50: Wallac Oy, Åbo, Finland. An X-ray of the lungs as well as a computed tomography (CT) and ultrasonic examination of the abdomen were also carried out. All blood chemistry analyses and radiographic examinations were repeated 1 month after termination of therapy and then when clinically indicated.

Registration of psychological well being

During each day of treatment the patients were asked to define their psychological well being as good, acceptable or poor. The psychological well being of the worst day during each treatment cycle is shown.

Criteria for response

Complete remission (CR) was defined as a complete disappearance of all radiological and biochemical evidence of tumor disease. Partial remission (PR) was attained when there was a decrease of at least 50% in the product of two perpendicular diameters of all measurable disease manifestations and a more than 50% decrease in the serum concentration of carcinoembryonic antigen (CEA), CA19-9 and CA50. Minor response (MR) was defined as a decrease in the product of two perpendicular diameters of at least one tumor lesion with 25%–50% and/or more than 50% decrease in the serum concentration of CEA, CA19-9 and/or CA50 and no increase (more than 25%) in any lesion. Stable disease (SD) was defined as no significant change (less than 25%) in the size of all measurable lesions and no significant change (less than 50%) in the serum concentrations of CEA, CA19-9 and CA50 for at least 3 months preceded by a progressive phase. Progressive disease (PD) was defined as an increase of more than 25% in the size of at least one measurable lesion and a more than 50% increase in the serum concentration of CEA, CA19-9 and/or CA50.

Isolation of peripheral blood mononulear cells (PBMC)

PBMC (lymphocytes and monocytes) were obtained by centrifugation of heparinized venous blood on a Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) gradient (density: 1.077 g/ml) and washed three times in Hank's TRIS solution [3].

Antibody-dependent cellular cytotoxicity

The cytotoxic activity was determined in an 18-h ^{51}Cr-release assay. After trypsinization of the target cells and testing for viability (trypan blue) the cells were labelled with 2.8 MBq sodium [^{51}Cr]chromate/ml cells (sp. act. = 8–18.5 GBq/mg chromium; Amersham International, UK) at 37 °C for 1 h. After three washes in full medium (Leibovitz medium 15, Gibco Ltd., Scotland, containing 10% fetal calf serum, antibiotics and 2 mmol L-glutamine) the cells were added to round-bottom wells of a 96-well microtiter plate (Nunc, Roskilde, Denmark). The effector cells were preincubated at 37 °C for 30 min and washed three times to remove mouse IgG absorbed in vivo and then added to give effector-to-target cell ratios of 50, 25, 12.5 and 6.25. mAb17-1A was added at the concentration of 10 μmg/ml. Supernatants were harvested by the Skatron Titer Tec System (Skatron A/S, Lierbyten, Norway) and counted in a gamma counter. Maximum isotope release was determined by incubation of the target cells with 5% Triton-X (Merck, Darmstadt, Germany). Spontaneous release was determined by incubation of ^{51}Cr-loaded target cells with medium alone. The percentage specific lysis was calculated by the formula

$$\text{Lysis (\%)} = \frac{\text{release in sample} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Results were then calculated as lytic units (LU)/10^6 effector cells, where 1 LU was defined as the number of PBMC required to obtain 30% specific lysis.

The lytic capacity in vivo should be related to the total number of available effector cells. During treatment, the total numbers of PBMC varied considerably, which has to be taken into consideration when estimating the lytic capacity of a patient. Thus, an absolute LU value was calculated on the basis of the total number of PBMC [17].

Quantitative analyses of human- anti-(mouse-antibodies) (HAMA) and anti-idiotypic antibodies (Ab_2)

The method for calculating the absolute concentration of HAMA and Ab_2 (IgG) has been described in detail elsewhere [21]. Briefly, polyclonal IgG antibody standards with specificity for mouse mAb17-1A (IgG2a, HAMA standard) and for the Fc part of mouse IgG2a [mAb425, anti-(epidermal growth factor receptor) antibody (Centocor); anti-isotypic IgG2a, antibody standard] were obtained by affinity chromatography purification of sera from patients treated with mAb17-1A and mAb425 respectively. A direct-binding enzyme-linked immunosorbent assay (ELISA) was used to determine IgG antibodies against mAb17-1A and the irrelevant isotype-matched mAb425 (IgG2a). The HAMA and anti-isotypic antibody standards were used to calculate the concentrations of antibodies against mAb17-1A and mAb425 respectively, using the linear regression model. The absolute concentration of Ab_2 was then calculated by subtracting the amount of anti-isotypic antibodies (anti-mAb425) from that of HAMA (anti-mAb17-1A).

mAb17-1A and GM-CSF-treated patients with metastatic colorectal carcinoma

Twenty patients with colorectal carcinoma have previously been treated with mAb17-1A and GM-CSF in the same dose schedule as in the present protocol [18]. Data on HAMA, Ab_2 and ADCC have been presented earlier [17, 21]. Some of these results are included in the present report for comparison. The long-term survival curve for these 20 patients is shown for the first time.

Statistical analyses

Analyses of differences between means were done by Student's *t*-test for paired and unpaired observations. Differences in distributions between groups were also tested with the Mann-Whitney *U*-test. Survival curves were generated by the life-table method. For comparing total blood cell numbers on day 10 of each cycle to those on day 10 of the first treatment cycle, analysis of variance with repeated measures was performed. Pairwise *post hoc* comparisons with adjustment according to Bonferroni [31] (three comparisons) were subsequently done. Calculations of antibody concentrations were made on logarithmically transformed values for distributional reasons and geometric means obtained from antilogarithms.

Results

Tumor response

One patient (patient 3) entered a partial remission. The case history of this patient is presented in detail below. Two other patients (patients 1 and 18) had stable disease for 7 and 4 months respectively. The median survival time of all patients was 8 months (range 2.5–31.5 months) (Fig. 1).

A man of 39 years (patient 3) presented with bleeding from the rectum and was subsequently operated upon

for a Dukes' stage D poorly differentiated adenocarcinoma of the rectum. Multiple excision biopsies from peritoneal metastases showed the presence of largely single tumor cells, small areas of extracellular mucin and a mild desmoplastic reaction with scanty lymphocytic infiltration in the stroma (Fig. 2a). The tumor cells showed the expression of GA73-3 (CO17-1A) by immunohistochemistry. Prior to the start of immunotherapy, abdominal CT and ultrasound scans failed to reveal any metastasis. Serum concentrations of CEA, CA19-9 and CA-50 were within the normal range. After four cycles of treatment the patient had a Karnowsky index of 100%. Three months after termination of therapy the patient underwent a second-look laparoscopy. At laparoscopy, only three nodules 2–5 mm in diameter, present in the rectovesical pouch, and one nodule on the serosa of the small intestine were resected. Except for the presence of these nodules, the rest of the abdomen was within the normal range. Two of the lesions showed tumor cells, well-formed glands, an intense desmoplastic reaction and moderate infiltration of mononuclear cells in the stroma (Fig. 2b). The patient was considered to have achieved a partial remission and two additional treatment cycles were given. One month after the last treatment cycle, a metastasis in the abdominal wall was diagnosed on fine-needle aspiration. The duration of the partial remission was 6.5 months.

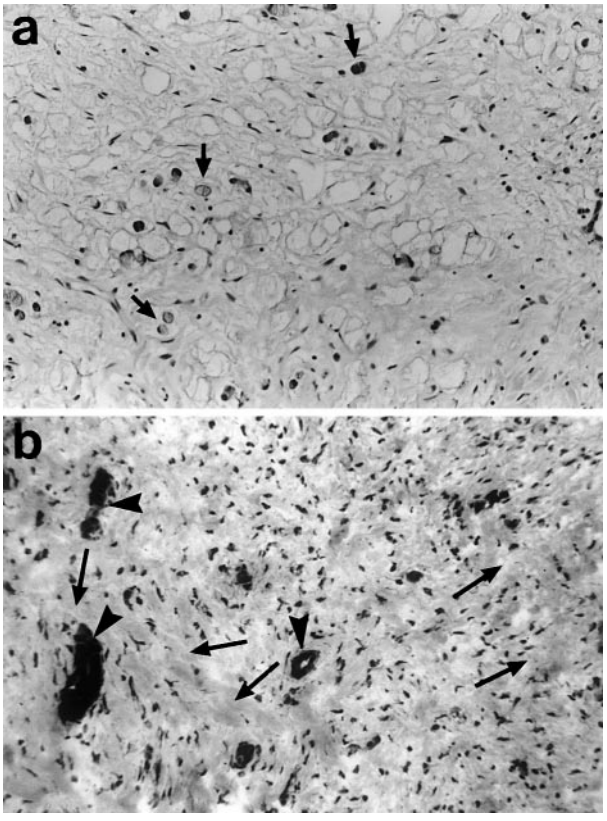

Fig. 2a, b Exercision biopsies of metastatic lesions (patient 3).**a** Pre-immunotherapy biopsy. Note single neoplastic cells (*arrows*) amidst pools of mucin and a mild desmoplastic reaction with scanty mononuclear cell infiltration in the stroma. **b** Post-immunotherapy biopsy (7 months later). Note the formation of tumor glands (*arrowheads*), an intense desmoplastic reaction (*arrows*) and the lymphocytic infiltration in the stroma (hematoxylin/eosin × 50)

Dosing of mAb17-1A

All patients received 400 mg mAb17-1A at the first cycle. Owing to type I skin reactions and ITAR, adjustments were made to the mAb dose in the subsequent cycles. At the second cycle, 16 patients received a full dose of mAb17-1A (400 mg) while 4 patients were given between 40 mg and 316 mg mAb17-1A. At the third cycle, 8 patients received 400 mg mAb, 8 patients 40 mg and 1 patient 16 mg. Three patients did not receive a third treatment cycle because of disease progression. At the fourth cycle, 4 patients were given 400 mg mAb17-1A, 1 patient 67 mg, 8 patients 40 mg and 1 patient 8 mg of the mAb. Six patients were not treated because of progressive disease.

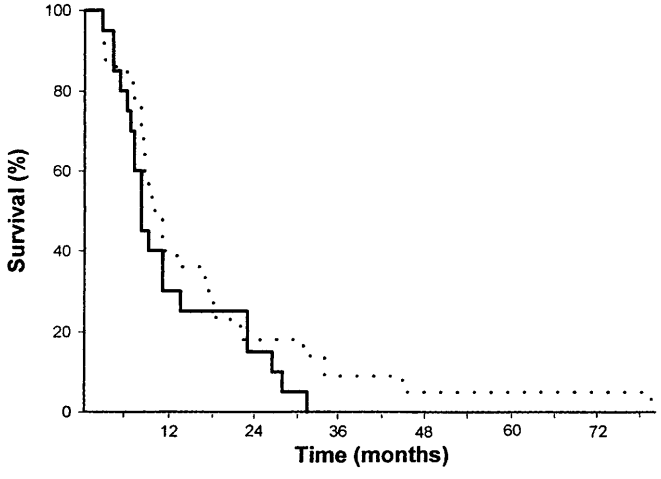

Fig. 1 Actuarial survival time of all patients with metastatic colorectal carcinoma treated with mAb17-1A/GM-CSF/IL-2(–) (median survival time 8 months), (n = 20). For comparison, the survival of patients treated with a combination of mAb17-1A/GM-CSF is included (. . .) (median survival time 11 months, n = 20; 18)

Dosing of IL-2 and GM-CSF

Only in 3 patients did the dose of IL-2 and GM-CSF have to be reduced. Side-effects that led to dose reduction were mainly fever, myalgia, malaise and dyspnea. In patients 15, 17 and 19 the IL-2 and GM-CSF doses were reduced by 25%–100%. Patient 17 did not receive IL-2 during cycles 3 and 4.

Blood cell counts

A significant increase in total white blood cell counts as well as in neutrophils, eosinophils, monocytes and lymphocytes was seen during all four treatment cycles

(Fig. 3). However, there was a clear tendency to a decreased increment in white blood cell counts and cell subsets as the number of treatment cycles increased. The decreased increment was statistically significant for white cell counts at cycle 4 and for neutrophils at cycles 3 and 4, comparing day 10 of the actual cycle to day 10 of cycle 1. The tendency was the same for eosinophils, monocytes and lymphocytes although the differences did not reach statistical significance. The cell counts returned to the pre-treatment level within 14–18 days after discontinuation of therapy.

All patients had a decrease in hemoglobin concentration during therapy. The mean decrease was 25%; 60% of the patients developed anemia (Hb <110 g/l) but only 1 patient required a blood transfusion. During all four treatment cycles a significant decrease (42 ± 6%, mean ± SEM; $P < 0.05$) in the platelet counts was noted at day 5 of the treatment cycle. The platelet counts had returned to the pre-treatment level before the subsequent cycle.

Blood chemistry analyses

A statistically significant decrease in serum albumin (20 ± 4%, mean ± SEM; $P < 0.01$) and serum calcium (8 ± 2%, $P < 0.05$) concentrations was noted during the treatment cycles as well as a significant increase (42 ± 8%, $P < 0.05$) in serum creatinine. Liver function tests remained normal.

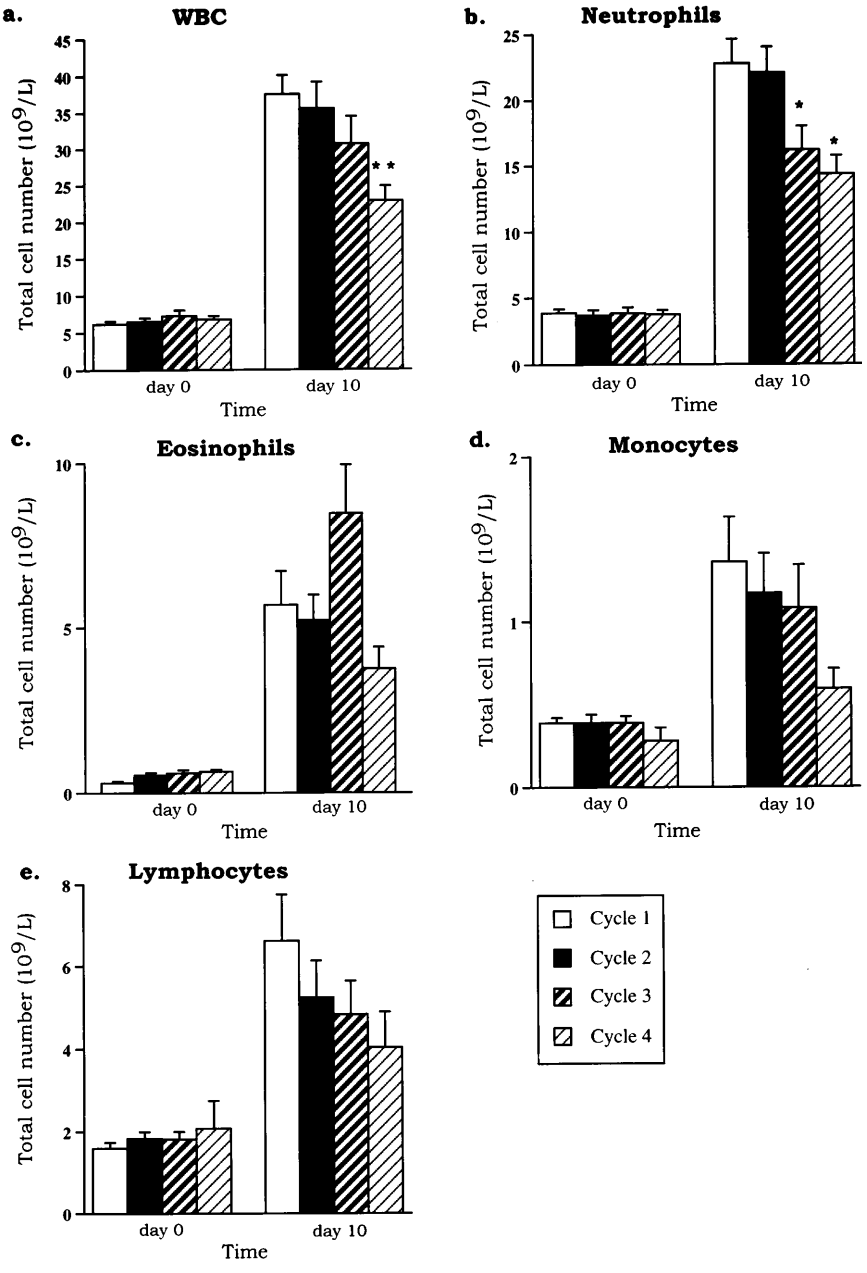

Fig. 3a–e Total number (mean ± SEM) of white blood cells (**a**), neutrophils (**b**), eosinophils (**c**), monocytes (**d**) and lymphocytes (**e**) on days 0 and 10 of the four treatment cycles. *($P < 0.05$) and **($P < 0.01$) indicate statistically significant differences comparing day 10 of the actual cycle with day 10 of cycle 1

Non-hematological side-effects

Types, frequencies and the severity of non-hematological side-effects are shown in Table 2. In the majority of the patients, side-effects were WHO grade II or less. Fatigue was the most prominent and frequent grade III side-effect (35%–60%).

mAb17-1A was given in planned doses to only 25% of patients. Dose reduction (see above) in response to allergic reactions was carried out in 20% of the patients at cycle 2, in 50% at cycle 3 and in 73% at cycle 4. One patient developed a grade II bronchospasm and urticaria and another patient a grade III hypotension and bronchospasm. Stopping the infusion of mAb17-1A and administration of fluid and antihistamine easily controlled the reactions.

Psychological well being

The acceptance of the treatment is shown in Table 3; 80% of the patients considered the side-effects to be acceptable. This figure did not change as the number of treatment cycles increased.

Comparison of immune reactions between patients treated with mAb17-1A/GM-CSF/IL-2 and mAb17-1A/GM-CSF

No patients had HAMA or Ab_2 before start of treatment. All patients in both treatment groups developed HAMA and Ab_2. However, 1 month after the last treatment cycle there was a highly statistically significant lower concentration of HAMA as well as of Ab_2 in the group receiving mAb17-1A/GM-CSF/IL-2 than in the group of patients who had received mAb17-1A/GM-CSF (Table 4). This statistical difference was also noted at other times when HAMA and Ab_2 were assayed, i.e. after the first and second treatment cycles respectively (data not shown).

Table 3 Psychological well being of patients with advanced colorectal carcinoma treated with a combination of mAb17-1A/GM-CSF/IL-2 in treatment cycles 1–4

Psychological well being	Number of patients			
	1 (n = 20)	2 (n = 20)	3 (n = 17)	4 (n = 14)
Good	6	6	5	3
Acceptable	11	10	9	9
Poor	3	4	3	2

During a 10-day treatment cycle with GM-CSF alone, ADCC increased. The maximum value was seen between days 5 and 7 of the treatment [17]. However, this was not the case for mAb17-1A/GM-CSF/IL-2-treated patients. When the maximum ADCC activities at days 5–7 of a treatment cycle were compared, a significantly lower ADCC activity was noted in the GM-CSF/IL-2 group than in the group of patients who had received only GM-CSF (Table 4).

Discussion

Twenty patients with advanced CRC were treated with a combination of mAb17-1A, GM-CSF and IL-2. One patient achieved a PR and 2 other patients were considered to have SD for 7 and 4 months respectively (overall CR + PR + MR + SD response rate, 15%). Side-effects were acceptable (WHO grade II or less) in the majority of patients.

The present trial is an extension of a previous study using mAb17-1A in combination with GM-CSF [18], a regimen that seemed to produce a better clinical response rate than mAb17-1A alone [22]. mAb17-1A alone in metastatic disease induced an overall response

Table 2 Number of patients and severity (WHO grading I–IV) of non-hematological side-effects for treatment cycles 1–4

Side-effects	Number of patients															
	1 (n = 20)				2 (n = 20)				3 (n = 16)				4 (n = 13)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Fever	1	16	3	0	3	12	5	0	4	7	2	0	2	3	5	0
Shivering	4	15	1	0	6	11	1	0	4	5	2	0	4	4	2	0
Myalgia/sternalgia	3	7	3	0	6	3	2	0	0	3	1	0	4	1	0	0
Nausea	6	11	0	0	6	11	0	0	5	7	0	0	1	8	0	0
Diarrhea	6	2	5	0	6	3	3	0	6	6	1	0	5	4	1	0
Dyspnea	0	5	10	0	0	10	7	0	2	4	9	0	1	4	8	0
Anorexia/weight loss	15	2	0	0	10	2	4	0	4	6	4	0	5	5	2	0
Fatigue	2	11	7	0	1	12	7	0	0	5	9	0	0	5	7	0
Rash, pruritus	3	13	0	0	2	13	0	0	4	9	0	0	4	7	0	0
Local irritation (injection sites)	16	0	0	0	18	0	0	0	16	0	0	0	12	0	0	0
Abdominal pains	3	8	1	0	4	2	3	0	6	2	0	0	3	0	0	0
Weight gain/edema	0	0	0	0	0	1	0	0	0	2	0	0	0	2	0	0
Hypotension	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Alopecia	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0

Table 4 Human anti-(mouse Ab) (*HAMA*), Ab_2 and antibody-dependent cellular cytotoxicity (*ADCC*) in colorectal carcinoma patients treated with mouse mAb17-1A in combination with GM-CSF/IL-2 compared to those treated with mAb17-1A together with GM-CSF only. Results are means ± SEM. HAMA and Ab_2 values 1 month after the last treatment cycle ($n = 20$) are shown. ADCC activity was determined during treatment cycle 1 or 2. It has previously been shown that ADCC activity during a 10-day GM-CSF treatment cycle has a maximum value during days 5–7. The maximum ADCC value on one of these three days is shown. *NS* not significant, *LU* lytic units

Cytokine regimen	HAMA (μg/ml)	Ab_2 (μg/ml)	ADCC (total LU)	
			Day 1 of the treatment cycle	Days 5, 6 or 7 of the treatment cycle
GM-CSF/IL-2	136.8 ± 46.4	20.4 ± 9.5	9.18 ± 2.35	6.97 ± 1.63
	$P < 0.01$	$P < 0.001$	NS	$P < 0.005$
GM-CSF	481.6 ± 90.7	356.0 ± 77.0	11.7 ± 1.82	19.08 ± 3.99

rate of 15% (11/71) with a median total survival of 11 months. When GM-CSF was added, the overall response in the same patient category was 30% (6/20) with a median survival time of 11 months (Fig. 1). To improve the clinical efficacy of mAb17-1A, GM-CSF and IL-2 were added to the therapeutic antibody, as in vitro data had indicated that preactivation of effector cells with GM-CSF and IL-2 rather than GM-CSF alone induced a significantly increased ADCC activity in the presence of mAb17-1A [14]. However, the clinical efficacy was not increased but a tendency to a lower response rate and impaired survival compared to mAb17-1A/GM-CSF-treated patients were observed.

mAb17-1A alone induced only minor side-effects and allergic reactions were rare [22]. When mAb17-1A and GM-CSF were combined, the majority of patients developed allergic reactions as the number of mAb infusions increased. Type I allergic skin reactions and ITAR meant that the mAb dose had to be reduced. At the second, third and fourth cycles the mAb dose was reduced in 30%, 83% and 91% of the patients respectively [18]. When IL-2 was added, the corresponding figures were 20%, 50% and 73%. Thus, the addition of IL-2 to mAb17-1A and GM-CSF did not increase the frequency and severity of allergic reactions against the mAb; there may even have been a tendency to reduction.

Schiller et al. reported no significant difference in the augmentation of ADCC activity when sequential treatment with IL-2 and GM-CSF was compared to simultaneous administration in cancer patients [26]. However, lymphokine-activated killer cell activity and the expression of CD16 on monocytes and lymphocytes and CD56 on lymphocytes were significantly lower in patients receiving IL-2 simultaneously with GM-CSF than in patients receiving the sequential treatment. Thus, simultaneous administration of GM-CSF and IL-2 might not be an optimal way to up-regulate immune functions in vivo. Our particular combination of mAb17-1A/GM-CSF/IL-2 seemed to induce some kind of immune suppression, as indicated by lower ADCC activity, HAMA production and Ab_2 response as well as a tendency to a lower frequency of allergic reactions than when mAb17-1A/GM-CSF was used. The reason for this is not clear.

An interesting observation was made in tumor biopsies of patient 3. After therapy an increase in the desmoplasia was seen. A desmoplastic reaction is considered to represent a host immune response against the tumor [27]. This finding is of interest as one of the effector functions of monoclonal antibodies has been suggested to be induction of a humoral and cellular idiotypic network response with the generation of tumor-specific antibodies and T cells [5]. The observation in this patient is in agreement with a recent report showing a gradual increase of T cells in the tumor lesions of mAb17-1A/GM-CSF-treated patients. $CD8^+$ T cells were localized around the tumor glands while $CD4^+$ T cells were scattered in the stroma [29].

During treatment, a statistically significant decrease in the increment of white blood cells and neutrophils was noted as time went on. This is in agreement with our earlier results in patients with colorectal carcinoma treated with a combination of mAb17-1A/GM-CSF, where the maximum total numbers of white blood cells, neutrophils, eosinofils and monocytes were significantly lower than the peak values of the previous cycles [19]. The decreased increment in blood cell subsets might be explained by development of anti-GM-CSF and anti-IL-2 antibodies since our earlier studies showed a relationship between the development of neutralizing anti-GM-CSF antibodies and the rise in white blood cell subsets [19, 33]. An alternative explanation might be a decrease in the stimulatory capacity and/or capacity of progenitor cells to respond by time.

In summary, the present treatment regimen combining mAb17-1A/GM-CSF/IL-2 seemed not to augment the antitumor effect of the mAb-based protocol but rather to hamper the clinical outcome. Moreover, addition of cytokines to the murine monoclonal antibody increased the immune response against the mAb and vigorously increased the adverse events profile, underlining the necessity of using humanized antibodies when combining them with agents aimed to augment immune effector functions.

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