Cancer Immunol Immunother (1993) 37: 264–270

Cancer Immunology Immunotherapy

Induction of an immune network cascade in cancer patients treated with monoclonal antibodies (ab_1)

I. May induction of ab_1-reactive T cells and anti-anti-idiotypic antibodies (ab_3) lead to tumor regression after mAb therapy?

J. Fagerberg[1], J-E. Frödin[1], H. Wigzell[2], H. Mellstedt[1]

[1] Department of Oncology (Radiumhemmet) & Immunological Research Laboratory, Karolinska Hospital, Stockholm, Sweden
[2] Department of Immunology, Karolinska Institute, Stockholm, Sweden

Received: 8 December 1992/Accepted 23 April 1993

Abstract. The antitumor effector functions of unconjugated monoclonal antibodies in cancer therapy are complex. Direct cytotoxic mechanisms such as antibody-dependent cellular cytotoxicity, complement-dependent cytolysis and apoptosis have been suggested. Induction of anti-idiotypic (ab_2) and anti-anti-idiotypic (ab_3) antibodies as well as T cell (T_2 and T_3 respectively) responses have also been proposed to be of clinical importance. In this study induction of an immune network cascade in patients with colorectal carcinoma, treated with mAb 17-1A (ab_1) was assessed. All patients developed anti-idiotypic antibodies (ab_2) of the IgG class after treatment with ab_1 and four of nine patients showed induction of mouse Ig reactive T cells [a proliferative response to $F(ab')_2$ fragments of ab_1]. Patients with such a T cell response developed anti-anti-idiotypic antibodies (ab_3), while those lacking the T cell reactivity failed to mount an ab_3 response. Three of four patients with a T cell response achieved a tumor response to mAb therapy. Thus, all responding patients belonged to the group of individuals developing ab_3. Induction of mAb(ab_1)-reactive T cells as well as an immune network cascade might be important antitumor effector functions of mAb and should be considered in the future design of mAb-based therapy protocols in cancer patients.

Key words: Monoclonal antibodies – Network response – Anti-idiotype antibodies – T cells – Colon carcinoma

Introduction

Tumor cells may differ from normal cells by the expression of various surface antigenic structures. Human tumor-specific antigens have been difficult to identify. Recently a gene encoding for an antigen on human melanoma cells was identified, which could code for a specific tumor antigen [4]. However, several well known oncofetal tumor-associated antigens (TAA) have been characterized. Molecules acting as TAA may be involved in cell differentiation and proliferation. Some are expressed at a high density during fetal stages, down-regulated and hardly detectable on normal mature cells but reexpressed at high concentrations on malignant cells [32]. Such antigens might be used as targets for immunotherapy.

Murine monoclonal antibodies (mAb) directed against TAA have been used for therapy of cancer patients. Most clinical experience has been obtained with unconjugated mAb [5, 19, 20, 28, 31, 34, 35]. Various direct antitumor effector functions of unconjugated mAb have been suggested, as antibody-dependent cellular cytotoxicity (ADCC) [1, 38], complement-dependent cytolysis [14] and apoptosis [42].

Other effector mechanisms associated with mAb treatment might also be of importance. In accordance with the idiotypic network hypothesis [21], the infused antibody (ab_1) may elicit an anti-idiotypic response against the idiotype of ab_1, antibodies (ab_2) as well as T cells (T_2). Parts of the variable regions of anti-idiotypic antibodies might resemble the epitope that ab_1 recognizes, i.e. the nominal antigen. The structural basis for the molecular mimicry may be due to shared primary sequences or expressed at the conformational level involving either V_L or V_H segments or composite V_L and V_H determinants [3, 6, 33]. ab_2 may subsequently induce a humoral and cellular anti-anti-idiotypic response, ab_3 and T_3 respectively, some of which may recognize the same epitope as ab_1. Whether an immune response against T cell receptor sequences of anti-idiotypic T cells can confer immunity on the nominal antigen is at present not known. However, an anti-anti-idiotypic response against ab_2 may be of importance for eradication of tumor cells.

In the present report induction of an immune (idiotypic) cascade in cancer patients treated with the unconjugated mAb 17-1A (ab_1) was analysed.

Correspondence to: H. Mellstedt, Department of Oncology (Radiumhemmet), Karolinska Hospital, S-104 01 Stockholm, Sweden

Materials and methods

Patients. At the Department of Oncology (Radiumhemmet), Karolinska Hospital, a clinical trial is in progress evaluating the antitumor effect of unconjugated mAb 17-1A in patients with advanced colorectal carcinoma [10, 31]. mAb 17-1A is a murine mAb (IgG_{2A}) for therapeutic use produced against the TAA, CO17-1A, a non-secreted 37-kDa glycoprotein [15]. Details of the treatment protocol, patient characteristics, response criteria and responses have been published previously [10]. This report includes nine patients treated with two to four courses of mAb 17-1A (400 mg at the first course and then 200 mg each course) with 6-week intervals. The mAb was given by an i. v. infusion together with isolated autologous mononuclear cells, preincubated with mAb 17-1A ex vivo [9]. There were five women and four men with a mean age of 60 years (range 32–81). The Karnowsky index was at least 80% in all patients at the start of mAb therapy. The patients were comparable with regard to prognostic factors and had comparable disease activity/tumor load [10]. Two patients (MD and WG) had undergone other treatments than surgery before mAb therapy. One had received chemotherapy and the other local radiotherapy. Informed consent was obtained from all patients.

Clinical response criteria. A clinical complete response was defined as a complete disappearance of all clinical, radiological and biochemical evidence of tumor disease. A partial response was obtained when there was a decrease of at least 50% in the product of two perpendicular diameters of all measurable disease manifestations and more than 50% decrease in the serum concentration of carcinoembryonic antigen (CEA), CA19-9 and CA50. A minor response 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 (>25%) in any lesion. Stable disease was defined as no significant change (<25%>) in the size of all measurable lesions and no significant change (<50%>) in the serum concentration of CEA, CA19-9 and CA50 for at least 3 months. Progressive disease was defined as an increase of more than 25% in the size of at least one measurable lesion and/or more than 50% increase in the serum concentration of CEA, CA19-9 and/or CA50.

Monoclonal antibodies. Mouse mAb 17-1A (IgG_{2A}), raised against the TAA CO17-1A [15], was used both for treatment of patients and in vitro tests. The chimeric (mouse × human) mAb 17-1A has the variable regions of mouse mAb 17-1A and the remaining part is derived from human IgG_1 [40]. Native and chimeric mAb 17-1A have the same antigenic specificity and affinity [36]. The mouse mAb BR55-2 (IgG_{2A}) against a glycolipid TAA expressed on human colon cancer cells was used for in vitro tests. mAb BR 55-2 does not cross-react with mAb 17-1A [39].

A monoclonal human IgG_1 (h-IgG_1) was used as a negative control in enzyme-linked immunosorbent assay (ELISA) inhibition tests. Isolation and purification of the antibody have been described in detail elsewhere [2]. A human anti-(mAb 17-1A) anti-idiotypic antibody (h-ab_2) (IgG_1) [37] was used as positive control in IgG ELISA.

Antibodies against F(ab′)$_2$ fragments of mAb 17-1A (ELISA). Antibodies against F(ab′)$_2$ fragments of mAb 17-1A were assayed in ELISA using flat-bottomed microtiter ELISA plates (Costar, Cambridge, Mass., USA) coated at 4° C overnight with 100 µl mAb 17-1A F(ab′)$_2$ fragments [43] (5 µg protein/ml) in 0.05 M carbonate buffer (pH 9.7). After three washes in PBS containing 0.05% Tween, the wells were blocked with bovine serum albumin (1%) (ISN Biochemicals, Cleveland, Ohio, USA) at 37° C for 1 h and washed three times. Diluted serum samples (1 : 60) were added for 2 h at 37° C. After washing, the wells were reacted for 2 h at 37° C with alkaline-phosphatase-conjugated goat anti-(human IgG) or anti-(human IgM) (1 : 1000) (Sigma, St Louis, Mo., USA). The wells were washed again and developed by adding 100 µl *p*-nitrophenyl phosphate (1 mg/ml) (Sigma) in 1 M diethanolamine buffer, pH 9.8, containing 0.5 mmol $MgCl_2$, and incubated for 20 min at room temperature. The absorbance was measured at 405 nm using an automatic ELISA reaader (Multi-Scan plus, Lab Systems, Helsinki, Finland). Results are expressed as mean absorbance of duplicate wells after subtraction of absorbance for background values. To assess the interplate variations h-ab_2 (10 µg/ml) and a patient serum sample (1 : 60) were used as positive controls in IgG and IgM ELISA respectively. As a negative control a serum sample (1 : 60) from a healthy donor was used. In 14 consecutive plates assaying IgG antibodies the absorbance was 1.276 ± 0.017 (mean ± SEM) for h-ab_2, 0.077 ± 0.006 (mean ± SEM) for the negative control and 0.044 ± 0.001 (mean ± SEM) for background wells. In 25 healthy controls the absorbance was 0.121 ± 0.013 (mean ± SEM). For IgM antibodies the absorbance in 17 consecutive plates was 0.581 ± 0.007 (mean ± SEM) for the positive patient serum, 0.060 ± 0.002 (mean ± SEM) for the negative control serum and 0.046 ± 0.001 (mean ± SEM) for background wells. In 25 healthy controls the absorbance was 0.078 ± 0.011 (mean ± SEM).

Anti-F(ab′)$_2$ antibodies were considered to be present when there was a three-fold or more rise in absorbance in comparison to the pretreatment value and absorbance exceeded the mean +2 SD of healthy controls (0.249 for IgG and 0.190 for IgM antibodies respectively, $n = 25$).

To determine the fraction of anti-idiotypic antibodies (ab_2), of the IgG class, binding to the variable regions of mAb 17-1A an inhibition ELISA was performed. Serum samples (1 : 60) were incubated with either chimeric mAb 17-1A (10 µg/ml) or an irrelevant human monoclonal control IgG_1 (h-IgG_1) antibody (10 µg/ml) at 4° C overnight. The samples were assayed in an ELISA as detailed above. The control antibody (h-IgG_1) induced an inhibition of up to 7% (mean +2SD = 7.7%, $n = 25$) of patient sera. Inhibition by the chimeric mAb 17-1A exceeding 10% was considered to indicate the presence of anti-idiotypic antibodies (ab_2) of the IgG class.

Anti-idiotypic antibodies of the IgM class (ELISA). Antibodies of the IgM class against chimeric mAb 17-1A were assayed in ELISA as described above except that plates were coated with chimeric mAb 17-1A (5 µg protein/ml). Results are expressed as the mean absorbance of duplicate wells after subtraction of absorbance for background values. A sample of a patients serum (1 : 60) and a serum sample (1 : 60) of a healthy control were used as positive and negative controls respectively. In four consecutive plates the absorbance was 0.582 ± 0.015 (mean ± SEM) for patient serum, 0.056 ± 0.028 (mean ± SEM) for the negative control and 0.052 ± 0.002 (mean ± SEM) for background wells. In 25 healthy controls the absorbance was 0.134 ± 0.020 (mean ± SEM). Anti-idiotypic antibodies (ab_2) of the IgM class were considered to be present when there was a three fold or more rise in the absorbance in comparison to the pretreatment value and the absorbance exceeded 0.336 (mean +2SD of healthy controls, $n = 25$).

Mixed hemadsorption assay for anti-anti-idiotypic antibodies (ab_3). A modified version of the mixed hemadsorption [8], described in detail elsewhere [11], was used to assess ab_3. Briefly, heat-inactivated serum (diluted 1 : 5) was incubated in flat-bottomed microtiter plates (Nunc, Roskilde, Denmark) with the human colorectal carcinoma cell line, SW1116, expressing the antigen CO17-1A. An indicator system consisting of fresh sheep red blood cells (SRBC) coated with monkey anti-SRBC antibodies (IgG) (National Bacteriological Laboratory, Stockholm, Sweden) and with sheep anti-(human IgG) antibodies (National Bacteriological Laboratory) was added. After incubation, tumor cells forming rosettes were counted. Tumor cells binding three or more SRBC were considered positive. A total of 200 tumor cells were counted in each well. The results are expressed as the percentage of cells forming rosettes. Serum from healthy donors gave $6.0 \pm 0.5\%$ rosettes (mean ± SEM, $n = 35$); serum was scored as positive if more than 15% rosettes were found. ab_3^+ serum did not bind to CO17-1A-negative tumor cell lines. The binding was inhibited by preincubation of the target cells with mAb 17-1A (ab_1) and incubation of the serum with human anti-(mAb 17-1A) ab_2 [37] as well as with a goat anti-(mAb 17-1A) ab_2 [18] (data not shown in the present report).

Isolation of lymphocytes. Lymphocytes were isolated from defibrinated venous blood by gelatin sedimentation and treatment with iron powder as described elsewhere [29]. Cell subsets were analysed by indirect immunofluorescence as previously described [27]. The antibodies indicated

Table 1. Anti-(mouse Ig) antibodies and anti-idiotypic antibodies (ab_2) of the IgG class in patients with colorectal carcinoma treated with mAb 17-1A

Patient	Binding of IgG antibodies to F(ab′)$_2$ fragments of mAb 17-1A (A_{405}) and inhibition (%) of binding by chimeric mAb 17-1A		
	Before treatment[a]	After treatment[b]	
	A_{405}	A_{405}	Inhibition[c] (%)
MD	0.061	0.677	25
TP	0.082	0.400	38
NH	0.057	0.351	48
UL	0.151	1.665	21
IP	0.070	0.781	46
LS	0.061	0.821	34
MW	0.114	1.307	27
IL	0.162	1.556	26
WG	0.198	1.385	25

[a] Serum samples drawn before mAb 17-1A treatment
[b] Serum samples were taken 4–6 weeks after treatment cycle 1 (patients MD, TP, NH, LS, MW, IL) or treatment cycle 2 (patients UL, IP, WG)
[c] Inhibition above 10% was considered to indicate the presence of ab_2

Table 2. Anti-(mouse Ig) antibodies and anti-idiotypic antibodies (ab_2) of IgM class in patients with colorectal carcinoma treated with mAb 17-1A

Patient	Binding to F(ab′)$_2$ fragments of mAb 17-1A		Binding to chimeric mAb 17-1A	
	Before treatment[a] A_{405}	After treatment[b] A_{405}	Before treatment[a] A_{405}	After treatment[b] A_{405}
MD	0.054	1.001	0.111	0.793
TP	0.046	0.098	0.576	0.566
NH	0.028	0.444	0.063	0.474
UL	0.164	0.592	0.094	0.433
IP	0.118	0.616	0.184	0.841
LS	0.053	0.585	0.053	1.128
MW	0.048	0.238	0.332	1.649
IL	0.101	0.315	0.125	0.406
WG	0.029	0.446	0.114	0.238

[a] Serum samples drawn before mAb 17-1A treatment
[b] Serum samples were taken 1–2 weeks after treatment cycle 1 except for patients MW and WG, where samples were taken 1–2 weeks after cycle 2

below were used in the first step and fluorescein-isothiocyanate-conjugated goat anti-(mouse IgG) antibodies (Becton-Dickinson, Mountain View, Calif., USA) or rabbit anti-(mouse IgG) F(ab′)$_2$ fragments (Dako, Copenhagen, Denmark) in the second step. Membrane fluorescence was examined in a Leitz Dialuxe 20 microscope with epi-illumination in transmitted ultraviolet and ordinary light ×1000 magnification; 400 cells were counted. The conjugate alone stained fewer than 0.5% cells. The purification process yielded a cell population of 81.7±2.1% $CD3^+$ cells (OKT3) (Ortho, Raritan, N. J., USA); 2.1±0.4% $CD20^+$ cells (B1) (Coulter, Hialeah, Fla., USA); 12.2±1.1% $CD11b^+$ cells (OKM1) (Ortho) and 11.0±1.5% $CD16^+$ cells (Leu11-b) (Becton-Dickinson). There was no difference in distribution of cell populations between patients and healthy controls (data not shown).

Proliferation assay. Purified mononuclear cells were resuspended in HEPES-buffered RPMI-1640 medium (Gibco, Paisley, Scotland) supplemented with antibiotics (100 IE penicillin and 100 µg streptomycin/ml), glutamine (2 mM) and 10% heat-inactivated normal human AB^+ serum. The cells (10^5/well) were cultured in 96-well round-bottomed microtiter plates (Nunc) at 37° C in humidified air with 5% CO_2 for 6 days. mAb 17-1A (whole antibody or F(ab′)$_2$ fragments) and polyclonal mouse IgG (Sigma) were added in increasing concentrations from 1 ng/ml to 10 µg/ml. Concanavalin A (Pharmacia, Uppsala, Sweden) was added at a concentration of 20 µg/ml and 40 µg/ml as a positive control. During the last 24 h of incubation 1 µCi/well [^{3}H]thymidine (specific activity 5 Ci/mmol) (Amersham, Amersham, UK) was added. The cells were harvested using an automatic Titertek cell harvester (Flow Lab, Irvine, Scotland). Radioactivity was measured in a liquid scintillation counter (LKB 1212, Rackbeta, Pharmacia-Wallac, Turku, Finland). The results are expressed as means of triplicates. A stimulation index (SI) was calculated for each triplicate set by dividing the mean ^{3}H (cpm) of stimulated cells with the mean value for unstimulated cells.

Statistics. Analyses of differences between means of non-parametric data were done by the Mann-Whitney *U*-test.

Results

Anti-idiotypic antibodies (ab_2)

Patients treated with mAb 17-1A (ab_1) developed anti-idiotypic antibodies (ab_2) of both the IgG and IgM classes. IgG antibodies were induced in all patients (Table 1). The post-treatment value for binding to F(ab′)$_2$ fragments of ab_1 (A_{405}) was more than three times the pretreatment value and clearly above the values of healthy controls (0.121±0.013, mean ± SEM, n = 25). The binding of all post-treatment sera could be inhibited by chimeric mAb 17-1A. Inhibition for the chimeric mAb 17-1A varied between 21% and 48%. The control antibody (h-IgG_1) induced an inhibition of up to 7% (mean +2SD = 7.7%, n = 25).

Seven of the nine patients also had IgM antibodies binding to chimeric mAb 17-1A after treatment (Table 2). Of the two ab_2-IgM-negative patients, one (WG) developed IgM antibodies against F(ab′)$_2$ fragments of mAb 17-1A. The other patient (TP) had a high pretreatment value for binding to chimeric mAb 17-1A. However, the absence of binding to F(ab′)$_2$ fragments from native mAb 17-1A indicates that the high value does not relate to preexisting IgM antibodies binding to the variable regions of mAb 17-1A. Moreover, patient TP did not develop detectable IgM antibodies against F(ab′)$_2$ fragments of mAb 17-1A after therapy (serum was checked at 1-week intervals after treatment cycles 1–3).

Six of the patients (MD, NH, LS, IL, UL, IP) had a primary and secondary humoral response indicating that mAb 17-1A acted as a thymus-dependent antigen. Binding curves from one of the patients are shown in Fig. 1. The remaining three patients (TP, WG, MW) had an anti-idiotypic IgG response before a detectable IgM response.

The humoral IgM response was further checked in ELISA using different whole antibodies for coating. One representative experiment is shown in Fig. 2. As can be seen the value for binding to mAb 17-1A (IgG_{2A}) was higher than to mAb BR55-2 (IgG_{2A}). Moreover, serum bound to chimeric mAb 17-1A but not to an irrelevant

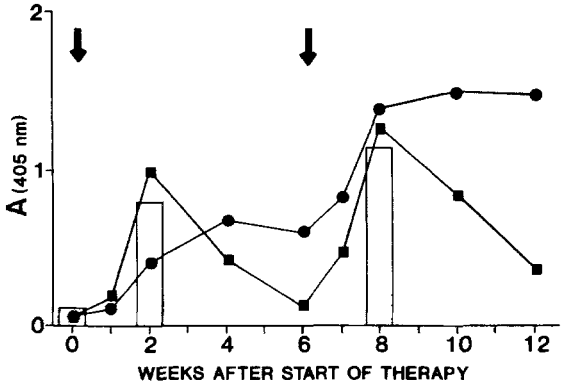

Fig. 1. Sera of patient MD tested in a direct ELISA (see Materials and methods) for antibodies against F(ab')2 fragments of mAb 17-1A and chimeric mAb 17-1A. ■, IgM antibodies against F(ab')2 fragments of mAb 17-1A; *columns*, IgM antibodies against chimeric mAb 17-1A; ●, IgG antibodies against F(ab')2 fragments of mAb 17-1A; ↓, infusion of mAb 17-1A

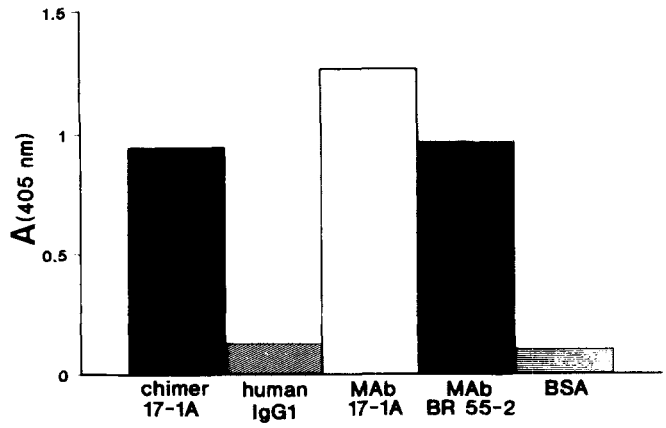

Fig. 2. Serum of patient MD after two cycles of mAb 17-1A infusions assayed for IgM antibodies in a direct ELISA (see Materials and methods) using wells coated with different antigens; 5 μg protein/ml was used for coating. *Chimer*, chimeric mAb 17-1A; *human IgG1*, h-IgG1; *BSA*, BSA only

human IgG1 (h-IgG1) or bovine-serum-albumin-coated wells.

T cell response against F(ab')2 fragments of ab1

To assess mouse Ig reactive T cells, a proliferation assay using mAb 17-1A (whole antibody and F(ab')2 fragments) or polyclonal mouse IgG as stimulant was done. In ten healthy control donors stimulation of purified mononuclear cells with F(ab')2 fragments of mAb 17-1A induced a maximal SI of 1.42 ± 0.44 (mean ± SD). Based on these results, an SI above 2.30 was considered a positive cellular response (mean value of healthy controls +2SD). This cut-off level for an SI value is comparable to those of other groups [22]. Three healthy donors had an SI value after stimulation with polyclonal mouse IgG of 5.43, 2.81 and 2.61 respectively but none of them showed a positive SI to mAb 17-1A, when either the whole antibody or F(ab')2 fragments were used. The background values (unstimulated cells) for controls (1.266 ± 205 cpm, mean ± SEM) as well as for patients (1.402 ± 279 cpm) were comparable. The concanavalin A response was somewhat lower in patients (16.380 ± 5.238 cpm) than in controls (24.376 ± 5.697 cpm), but the difference was not statistically significant.

Patients were tested repeatedly during therapy-free intervals. Four (TP, NH, UL, MW) of the nine patients developed cells that proliferated in response to F(ab')2 fragments of mAb 17-1A, the whole mAb 17-1A molecule and polyclonal mouse IgG (Table 3). In one patient (UL) the response to F(ab')2 fragments of mAb 17-1A was remarkably high. Two of the patients exhibited a cellular response after one infusion of mAb 17-1A and the other two after two and three infusions respectively. One patient (IP) had a proliferative response to polyclonal mouse IgG but not to F(ab')2 fragments of mAb 17-1A, before as well as after treatment, indicating a response to Fc-γ regions of mouse IgG. This polyclonal mouse IgG response also increased after therapy. In four other patients no positive SI values were recorded during repeated testing. The optimal

Table 3. T cells responding to F(ab')2 fragments of ab1 in peripheral blood of patients with colorectal carcinoma treated with mAb 17-1A (ab1)

Patient	Maximum stimulation index (SI)[a]						
	Before treatment			After treatment			
	Whole mAb 17-1A	F(ab')2 fragments of mAb 17-1A	Polyclonal whole mouse IgG	No of mAb 17-1A inf.	Whole mAb 17-1A	F(ab')2 fragments of mAb 17-1A	Polyclonal whole mouse IgG
MD	ND	ND	ND	2	1.62	ND	1.39
TP	1.29	1.32	1.41	1	3.60[b]	**2.71**[c]	4.69[c]
NH	1.11	0.95	1.37	2	3.35[b]	**2.88**[d]	3.06[b]
UL	1.42	0.69	1.60	1	5.36[b]	**20.33**[b]	9.80[c]
IP	4.35[c]	2.20	3.97[d]	2	2.06	1.86	18.22[b]
LS	2.17	1.08	0.89	2	1.44	1.01	0.86
MW	0.91	1.51	0.78	3	7.25[d]	**4.92**[d]	7.79[e]
IL	1.60	ND	1.49	2	0.79	ND	0.72
WG	1.43	ND	1.10	1	1.40	ND	1.43

[a] The highest SI value is shown, obtained after stimulation with different concentrations of the antibodies (1 ng/ml – 10 μg/ml). The individual optimal antibody concentration in a positive test is: [b] 10 μg/ml, [c] 1 μg/ml, [d] 0.1 μg/ml and [e] 1 ng/ml. Values in bold type indicate the presence of F(ab')2-reactive T cells. ND, Not done

Table 4. Anti-anti-idiotypic antibodies (ab_3) in serum of patients with colorectal carcinoma treated with mAb 17-1A (ab_1)

Patient	Rosettes (%)	
	Before treatment	After treatment
MD	–[a]	–
TP	–	20
NH	–	21
UL	–	37
IP	–	–
LS	–	–
MW	–	74
IL	40	70
WG	–	–

[a] –, Negative; serum was scored as negative if 15% or fewer rosettes were found. ab_3^+ serum did not bind to CO17-1A-negative tumor cell lines. The binding was inhibited by preincubation of the target cells with mAb 17-1A (ab_1) and by incubation of the serum with a human anti-(mAb 17-1A) ab_2 as well as with a goat anti-(mAb 17-1A) ab_2 [11]

Ig concentration inducing maximum cellular response varied between individuals. Stimulation with 1 μg/ml or 10 μg/ml generally induced the highest SI value.

Anti-anti-idiotypic antibodies (ab_3)

After treatment with mAb 17-1A four patients developed antibodies against the human colorectal carcinoma cell line SW1116 expressing the antigen CO17-1A (Table 4). The time until ab_3 was detectable varied between 3 weeks and 26 weeks. ab_3 was found in serum for 1 year after the start of therapy in three patients (TP, UL, MW) and in one patient (NH) for 4 years. Patients with no detectable ab_3 were followed for 19–62 weeks after therapy, but no positive sera were scored. One patient (IL) had antibodies against SW1116 before therapy, and the titer rose after treatment. This patient was followed for 8 months, during which period the serum remained positive. The existence of auto-antitumor antibodies has been described previously [11, 18, 26].

The immune network response in relation to tumor response

Interestingly, induction of ab_3 was only noted in those patients who developed a cellular response to ab_1 (Table 5). In the exceptional patient IL, ab_3 was not induced by the therapy but was present before treatment and no induction of T cells reactive with mAb 17-1A could be detected. Among the four patients who developed ab_3 and T cells responding to $F(ab')_2$ fragments of ab_1, three (TP, NH, MW) also achieved a tumor response (CR or MR) after mAb 17-1A therapy.

Five patients failed to develop ab_3 or a detectable T cell response to ab_1 as a result of the treatment. None of these patients showed any signs of tumor reduction induced by the mAb 17-1A therapy.

Discussion

Unconjugated mAb might inhibit growth of malignant tumors both in animal models [12, 16] and in patients [5, 19, 20, 28, 31, 34, 35]. Direct antitumor effector functions mediated by the mAb are suggested to be ADCC, complement-dependent cytolysis or apoptosis. Induction of an idiotypic network response has also been proposed to mediate tumor cell killing, as an indirect effector mechanism [23]. Anti-idiotypic antibodies (ab_2) [7, 11, 17, 23, 37, 41] and anti-anti-idiotypic antibodies (ab_3) [7, 11, 44] have been induced by mAb (ab_1) treatment in cancer patients and been suggested to be of benefit for the patients [11, 23]. Little is known about the induction of an idiotypic T cell response in humans. Kosmas et al. presented data indicating the induction of idiotype(ab_1)-reactive T cells after radioimmunotherapy with murine mAb [24]. Lanzavecchia et al. showed that after treatment with mouse mAb, T cells recognizing isotypic determinants of the mouse immunoglobulin could be detected [25].

In the present study all patients developed IgG antibodies binding to $F(ab')_2$ fragments of mAb 17-1A (ab_1), i.e. anti-(mouse Ig) antibodies, after therapy. Part (21%–48%) of the binding activity to $F(ab')_2$ fragments of mAb 17-1A was inhibitable by the chimeric mAb 17-1A, but not with

Table 5. Induction of an idiotype network response in patients with colorectal carcinoma treated with mAb 17-1A (ab_1) and relation to the clinical effect

Patient	Humoral response:		Cellular response: ab_1-reactive T cells	Clinical response to treatment[b]	Survival[c] (months)
	ab_2[a]	ab_3			
MD	+	–	–	PD	5
TP	+	+	+	MR	12
NH	+	+	+	CR	86 + NED
UL	+	+	+	PD	12
IP	+	–	–	PD	11
LS	+	–	–	PD	7
MW	+	+	+	MR	19
IL	+	(+)[d]	–	PD	18
WG	+	–	–	SD	28

[a] IgG antibodies

[b] Response criteria (CR, complete remission; MR, minor response; SD, stable disease; PD, progressive disease) and responding patients have been described in detail elsewhere [10]

[c] Time from start of mAb 17-1A treatment. NED, no evidence of disease

[d] Antibodies against a CO17-1A-expressing tumor cell line were not induced by ab_1 therapy but present before treatment

an irrelevant human control IgG_1 antibody, strongly indicating that there were antibodies binding to the variable regions of mAb 7-1A, i.e. true anti-idiotypic antibodies (ab_2). In seven of nine patients, anti-idiotypic IgM antibodies could be detected after treatment. The high frequency of anti-isotypic and anti-idiotypic antibodies after ab_1 therapy is in accordance with a previous report where 95% (41/43) of the patients developed ab_2 [11].

Induction of ab_1-reactive T cells proliferating in response to $F(ab')_2$ fragments of ab_1 was noticed in only four of the patients (44%). The fine specificity of the T cell response in this study can not at present be established. The immunotopes are located within the $F(ab')_2$ region. However, it can not be determined whether the T cell response was against species-restricted, isotypic and/or idiotypic determinants. In patient UL the high SI value in response to $F(ab')_2$ fragments favors arguments for a predominance of an idiotypic T cell response. To answer the question whether the T cell response was against isotypic and/or idiotypic determinants the chimeric mAb 17-1A would have been the best one to use in the proliferation assay. Unfortunately, the antibody was not available in sufficient amounts when the tests in the present report were performed.

It has previously been shown by Lanzavecchia et al. that patients treated with mouse mAb developed $CD4^+$ T cells reacting with isotypic determinants within the $F(ab')_2$ region. Such T cells could lyse tumor cells in vitro that had bound, taken up and processed the specific mouse mAb [25]. T cells recognizing mouse immunoglobulin might accordingly be of therapeutic benefit in the present system as well. When a mAb that has previously been given to a patient is readministered, the tumor-bound mAb may be internalized, processed and presented together with major histocompatibility complex, whereafter the mouse Ig-specific T cells might kill the mAb-modified tumor cells. mAb 17-1A bound to tumor cells can be internalized [45], indicating that this mechanism of action might also be functioning in the present therapeutic situation. In this study three of four patients with T cells recognizing $F(ab')_2$ fragments of mAb 17-1A had a tumor response (CR or MR) after treatment while none of five patients without a T cell response showed any tumor reduction.

If induction of an idiotypic cascade might lead to a secondary effector function of the administered anti-TAA mAb, antibodies (ab_3) and/or T cells (T_3) recognizing the nominal antigen (TAA) should be induced. Patients developing ab_3 after mAb 17-1A therapy have been reported to survive significantly longer than those without ab_3 and a statistically significant positive correlation has been found between the presence of ab_3 and tumor response [11]. In the present study we have only analyzed the humoral part of the anti-anti-idiotypic response. Among the nine patients, four developed ab_3 and one further patient had detectable antibodies binding to SW1116 tumor cells before therapy. Tumor regression (complete remision or minor response) was noted in three of these five patients while none of four patients lacking ab_3 had any tumor response. Interestingly, only patients who developed mouse Ig reactive T cells showed induction of ab_3. Thus, responding patients had both a T cell response against ab_1 and a humoral anti-anti-idiotypic response. The relation between ab_1-responding T cells and induction of ab_3 is at present not clear but needs further study.

It has been argued that tumor cell destruction is mainly mediated by T cells [13]. If this is true, induction of T cells (T_3) should be of great importance in comparison to the humoral response (ab_3). We have preliminary results indicating that patients treated with mAb might mount an ab_3 response, with and without the induction of T_3 cells, similar to the findings of the present study, but only patients who developed T_3 cells showed a tumor reduction (to be published). This is in accordance with our previous findings that patients with a tumor response to mAb therapy were those who developed a positive delayed-type hypersensitivity reaction to a human ab_2, i.e. patients with T_3 cells [31].

The present and previous reports support the suggestion that induction of an idiotypic cascade in patients treated with unconjugated mAb might lead to a clinically important antitumor effect. T cells directed against mouse Ig structures other than idiotypes might likewise be of value during such a treatment with antitumor mAb.

Acknowledgements. This study was approved by the Ethical Committee of the Karolinska Institute and supported by grants from the Swedish Cancer Society, the Cancer Society in Stockholm, the King Gustav V Jubilee Fund, the Elina Andersen Foundation and the Karolinska Institute Foundations. We thank Professor Hilary Koprowski and Professor Zenon Steplewski for providing us with mouse and chimeric mAb 17-1A and mAb BR 55-2. We thank Ms. Birgitta Hagström and Ms. Jutta Andersson for expert technical assistance and Ms. Marie Lindroos for excellent typing of the manuscript.

References

1. Adams DO, Hall T, Steplewski Z, Koprowski H (1984) Tumors undergoing rejection induced by monoclonal antibodies of the IgG_{2A} isotype containing increased numbers of macrophages activated for distinctive form of antibody dependent cytolysis. Proc Natl Acad Sci USA 81: 3506
2. Bergenbrandt S, Österborg A, Holm G, Mellstedt H, Lefvert AK (1991) Anti-idiotypic antibodies in patients with monoclonal gammopathies: relation to the tumor load. Br J Haematol 78: 66
3. Bruck C, Sung Co M, Slaoui M, Gaulton GN, Smith T, Fields BN, Mullins JI, Greene MI (1986) Nucleic acid sequence of an internal image-bearing monoclonal anti-idiotype and its comparision to the sequence of the external antigen. Proc Natl Acad Sci USA 83: 6578
4. Bruggen van der P, Traversari C, Chomez P, Lurquin C, Plaen de E, Eynde van den B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254: 1643
5. Cheung NKV, Lazarus H, Miraldi FD, Abramowsky CR, Kallick S, Saarinen UM, Spitzer T, Stransjord SE, Coccia PF, Berger NA (1987) Ganglioside GD2 specific monoclonal antibody 3F8: a phase-I study in patients with neuroblastoma and malignant melanoma. J Clin Oncol 5: 1430
6. Cleave van VH, Naeve CW, Metzger DW (1988) Do antibodies recognize amino acid side chains of protein antigens independently of the carbon backbone? J Exp Med 167: 1841
7. Courtenay-Luck NS, Epenetos AA, Sivolapenko GB, Barkans JR, Larche M, Ritter MA (1988) Development of anti-idiotypic antibodies against tumor antigens and auto-antigens in ovarian cancer patients treated intraperitoneally with mouse monoclonal antibodies. Lancet II: 894

8. Fagreus A, Espmark JÅ, Jonsson J (1965) Mixed haemadsorption: a mixed anti-globulin reaction applied to antigens on a glass surface. Immunology 9: 161
9. Frödin JE, Pihlstedt P, Lefvert AK, Mellstedt H (1987) Isolation and characterization of autologous blood mononuclear cells used for auto-infusion together with monoclonal antibodies in tumor treatment. Hybridoma 6: 555
10. Frödin JE, Harmenberg U, Biberfeld P, Christensson B, Lefvert AK, Rieger Å, Shetye J, Wahren B, Mellstedt H (1988) Clinical effects of monoclonal antibodies (MAb 17-1A) in patients with metastatic colorectal carcinoma. Hybridoma 7: 309
11. Frödin JE, Faxas ME, Hagström B, Lefvert AK, Masucci G, Nilsson B, Steinitz M, Unger P, Mellstedt H (1991) Induction of anti-idiotypic (ab_2) and anti-anti-idiotypic (ab_3) antibodies in patients treated with the mouse monoclonal antibody 17-1A (ab_1). Relation to the clinical outcome – an important antitumoral effector function? Hybridoma 10: 421
12. Hearing VJ, Leong SPL, Vieira WD, Law LW (1991) Suppression of established pulmonary metastases by murine melanoma-specific monoclonal antibodies. Int J Cancer 47: 148
13. Hellström K, Hellström I (1990) Antitumor vaccines. I. Why cancer vaccines? In: New generation vaccines. GC Woodrow, MM Levine (eds) Marcel-Dekker, New York, p 855
14. Herlyn DM, Koprowski H (1981) Monoclonal anticolon carcinoma antibodies in complement-dependent cytotoxicity. Int J Cancer 27: 769
15. Herlyn DM, Steplewski Z, Herlyn D, Koprowski H (1979) Colorectal carcinoma-specific antigen detection by means of monoclonal antibodies. Proc Natl Acad Sci USA 76: 1438
16. Herlyn DM, Steplewski Z, Herlyn MF, Koprowski H (1980) Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. Cancer Res 40: 717
17. Herlyn DM, Sears H, Iliopoulos D, Lubeck M, Douillard JY, Sindelar W, Tempero M, Mellstedt H, Maher M, Koprowsky H (1986) Anti-idiotypic antibodies to monoclonal antibody CO17-1A. Hybridoma 5: 51
18. Herlyn DM, Wettendorff M, Schmoll E, Iliopoulos D, Schedel I, Dreikhausen U, Raab R, Ross AH, Jaksche H, Scriba M, Koprowski H (1987) Anti-idiotype immunization of cancer patients: modulation of the immune response. Proc Natl Acad Sci USA 84: 8055
19. Houghton AN, Mintzner D, Cordon-Cardo C, Welt S, Fliegel F, Vadham S, Carswell E, Melamed MR, Oettgen HF, Old LY (1985) Mouse monoclonal IgG3 antibody detecting 6D3 ganglioside: a phase I trial in patients with malignant melanoma. Proc Natl Acad Sci USA 82: 1242
20. Irie RF, Morton DL (1986) Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside GD2. Proc Natl Acad Sci USA 83: 8694
21. Jerne NK (1974) Towards a network theory of the immune system. Ann Immunol 125: 373
22. Kabilan L, Troye-Blomberg M, Perlmann H, Andersson G, Högh B, Petersen E, Björkman A, Perlmann P (1988) T-cell epitopes in Pf155/RESA, a major candidate for a *Plasmodium falciparum* malaria vaccine. Proc Natl Acad Sci USA 85: 5659
23. Koprowski H, Herlyn D, Lubeck M, DeFreitas E, Sears HF (1984) Human anti-idiotype antibodies in cancer patients: is the modulation of the immune response beneficial for the patient? Proc Natl Acad Sci USA 81: 216
24. Kosmas C, Epenetos AA, Courtenay-Luck NS (1991) Patients receiving murine monoclonal antibody therapy for malignancy develop T cells that proliferate in vitro in response to these antibodies as antigens. Br J Cancer 64: 492
25. Lanzavecchia A, Abrignani S, Scheidegger D, Obrist R, Dörken B, Moldenhauer G (1988) Antibodies as antigens. The use of mouse monoclonal antibodies to focus human T cells against selected targets. J Exp Med 167: 345
26. Lehmann HP, Zwicky C, Waibel R, Stahel R (1992) Tumor-antigen-specific humoral immune response of animals to anti-idiotypic antibodies and comparative serological analysis of patients with small-cell lung carcinoma. Int J Cancer 50: 86
27. Lindemalm C, Mellstedt H, Biberfeld P, Björkholm M, Christensson B, Holm G, Johansson B, Sundblad R (1983) Blood and lymph node T lymphocyte subsets in non-Hodgkin lymphomas. Scand J Haematol 30: 68
28. LoBuglio AF, Saleh MN, Lee J, Khazaeci MB, Carrano R, Holden H, Wheeler RH (1988) Phase I trial of multiple large doses of murine monoclonal antibody CO17-1A. I. Clinical aspects. J Natl Cancer Inst 80: 932
29. Mellstedt H, Holm G (1973) In vitro studies of lymphocytes from patients with plasma cell myeloma. I. Stimulation by mitogens and cytotoxic activities. Clin Exp Immunol 15: 309
30. Mellstedt H, Frödin JE, Biberfeld P, Fagerberg J, Giscombe R, Hernandez A, Masucci G, Li SL, Steinitz M (1991) Patients treated with a monoclonal antibody (ab_1) to the colorectal carcinoma antigen 17-1A develop a cellular response (DTH) to "the internal image of the antigen" (ab_2). Int J Cancer 48: 344
31. Mellstedt H, Frödin JE, Masucci G, Ragnhammar P, Fagerberg J, Hjelm AL, Shetye J, Wersäll P, Österborg A (1991) The therapeutic use of monoclonal antibodies in colorectal carcinoma. Semin Oncol 18: 462
32. Neville AM, Gusterson BA (1985) Monoclonal antibodies and human tumours: pathological and clinical aspects. Eur J Cancer Clin Oncol 21: 355
33. Raychaudhuri S, Kang C-Y, Kaveri S-V, Kieber-Emmons T, Köhler H (1990) Tumor idiotype vaccines. VII. Analysis and correlation of structural, idiotypic, and biologic properties of protective and non-protective Ab_2. J Immunol 145: 760
34. Sears HF, Mattis J, Herlyn D, Hayry P, Atkinson B, Ermst C, Steplewski Z, Koprowski H (1982) Phase I clinical trial of monoclonal antibody treatment of gastrointestinal tumors. Lancet I: 762
35. Sears HF, Herlyn D, Steplewski Z, Koprowski H (1985) Phase II clinical trial of a murine monoclonal antibody cytotoxic for gastrointestinal adenocarcinoma. Cancer Res 45: 5910
36. Shaw D, Khazaeli MB, Suu L, Ghrayeb J, Dadonna P, McKinney S, Lobuglio A (1987) Characterization of a mouse/human chimeric monoclonal antibody (17-1A) to a colon cancer tumor-associated antigen. J Immunol 138: 4534
37. Steinitz M, Tamir S, Frödin JE, Lefvert AK, Mellstedt H (1988) Human monoclonal anti-idiotypic antibodies. I. Establishment of immortalized cell lines from a tumor patient treated with mouse monoclonal antibodies. J Immunol 141: 3516
38. Steplewski Z, Lubeck MD, Koprowski H (1983) Human macrophages armed with murine IgG2a anti-tumor immunoglobulins destroy human cancer cells. Science 221: 865
39. Steplweski Z, Blaszezyle M, Herlyn D, Herlyn M, Koprowski H (1985) Effector cells in ADCC with antibreast cancer monoclonal antibodies: In: Monoclonal antibodies and breast cancer. Nijhoff, Boston, p 134
40. Sun L, Curtis P, Rabrowicz-Szulezyska E, Ghrayeb J, Morrison S, Chang N, Koprowski H (1986) Chimeric antibodies with 17-1A derived variable and human constant regions. Hybridoma 5 [Suppl 1]: 17
41. Traub UC, DeJager RL, Primus FJ, Losman M, Goldenberg DM (1988) Anti-idiotype antibodies in cancer patients receiving monoclonal antibody to carcinoembryonic antigen. Cancer Res 48: 4002
42. Trauth BC, Klas C, Peters AMJ, Matzku S, Möller P, Falk W, Debatin KM, Krammer PH (1989) Monoclonal antibody mediated tumor regression by induction of apoptosis. Science 245: 301
43. Waller M, Curry N, Mallory J (1968) Immunochemical and serological studies of enzymatically fractionated human IgG immunoglobulins. I. Hydrolysis with pepsin, papain, ficin and bromelin. Immunochemistry 5: 577
44. Wettendorff M, Iliopoulos D, Tempero M, Kay D, DeFreitas E, Koprowski H, Herlyn D (1989) Idiotypic cascades in cancer patients treated with monoclonal antibody CO17-1A. Proc Natl Acad Sci USA 86: 3787
45. Woo DV, Li D, Mattis JA, Steplewski Z (1989) Selective chromosomal damage and cytotoxicitiy of ^{125}I-labeled monoclonal antibody 17-1a in human cancer cells. Cancer Res 49: 2952