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Immunology

Human anti-idiotype antibodies in cancer patients: Is the modulation of the immune response beneficial for the patient?

(internal image of antigen/gastrointestinal cancer/monoclonal antibody)

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ABSTRACT **Inoculation of human subjects with mouse monoclonal antibody results in the production of anti-idiotype antibody that reacts with the binding site of the monoclonal antibody. This reaction is hapten-inhibited, suggesting that an internal image of the antigen is produced by the anti-idiotype response. The anti-idiotype antibody isolated from sera of three patients showed significant crossreactivity. Patients who developed the anti-idiotype antibody improved clinically and had long remission from their disease. The possible presence of the internal image of cancer antigen on the human immunoglobulin molecule may change the conditions under which the immune system reacts to the tumor antigen and may open new approaches to the control of tumor growth.**

The treatment of human leukemias with mouse monoclonal antibody (mAb) directed against antigen(s) present on leukemia cells results in a transient destruction of the leukemia cells (1), observed almost immediately after administration of the mAb (2). Sera of some patients who received anti-Leu 1 mAb for treatment of T-cell leukemia inhibited binding of fluoresceinated mAb to T leukemia cells and were considered to contain an anti-idiotype (anti-Id) antibody. This was also observed in sera of renal transplant patients who received an OKT3 anti-T-cell mouse mAb (3). It has been suggested that binding of anti-Id to the mAb may prevent the antitumor activity of the mAb (1). This may be true in cases when direct action of mAb on cancer cells occurs (2). However, when no immediate antitumor effect of mAb is observed and when induction of tumor cell destruction occurs only a long time after the administration of mAb (4), it may be that an immune response of the host to his tumor is induced by mAb treatment.

Jerne has postulated a network of interacting antibody molecules and lymphocytes in the immune system in which idiotypes of antibody molecules are recognized by anti-idiotypic antibodies and cells (5). Within the set of anti-idiotypic antibodies could be expected to be those that were directly complementary to the parotope—i.e., the antigen binding site of the idiotypic antibody. The binding of such anti-idiotypic antibodies to the idiotypes has in fact been shown to be inhibitable by the antigen. This has led to the concept that such anti-idiotypic antibodies are internal images of the antigen. It was therefore predicted that priming an animal with anti-Id rather than with an antigen would be possible, and this, in fact, has been successfully achieved (6). The first physiological evidence that anti-Id could mimic the action of an antigen was obtained with anti-Ids against insulin (7). Direct evidence for the beneficial effect of an anti-Id antibody in infectious diseases has recently been found in mice primed with anti-Id in the absence of antigen that appeared immunized against *Trypanosoma rhodesiense* after such treatment (8). When these animals were challenged with *T. rhodesiense*, specific idiotype appeared in all animals, some of which had the specific idiotype even before challenge.

In the present paper, we describe the development of anti-Id in sera of patients with gastrointestinal cancer who were treated with mouse mAb. Furthermore, we show that binding between the anti-Id and mAb can be inhibited by hapten. This suggests that the anti-Id is the internal image of the antigen expressed by the cancer cells. Finally, we discuss these findings in relation to the outcome of mAb treatment of gastrointestinal cancer.

MATERIALS AND METHODS

mAbs. Mouse mAbs 17-1A, $C_4$2032, and $C_4$1472, which bind to human gastrointestinal cancer cells, have been described elsewhere (9, 10). mAb $C_4$2032 is specific for colorectal carcinoma (CRC)-associated antigen(s) of M_r 180,000, 160,000, 50,000, and 40,000. mAb $C_4$1472 (IgG2a) is specific for a CRC-associated antigen of M_r 50,000. The A5C3 mAb against hepatitis virus has been described (11). mAb 17-1A (IgG2a, κ light chain) and $C_4$2032 (IgG2a, κ light chain) were purified from ascites obtained from hybridoma-bearing mice by affinity chromatography on a protein A-Sepharose column (Pharmacia) as described by Ey *et al.* (12).

Colorectal Cancer Extract. The 3 M KCl extract of SW-1222 cells, a cell line derived from colorectal cancer, has been described (13). Since this preparation binds mAb 17-1A, it seems likely that the material containing the antigen is in soluble form.

Patients. All patients had metastatic or recurrent gastrointestinal adenocarcinoma and were injected systemically with one dose of a purified sterile pyrogen-free preparation of mAb 17-1A (9) concentrated from ascites fluid of BALB/c mice (14). Of 9 patients who received 192 mg or less of mAb, 7 developed anti-mouse globulin antibodies (10). Of 20 patients who received 200–1,000 mg of mAb, 3 developed anti-mouse globulin antibodies (10). Sera of 3 patients of the first group who developed anti-mouse globulin antibodies (nos. 07, 08, and 09) (10) and of 2 patients of the second group (nos. 14 and 23) were either screened or processed for isolation of anti-Id antibodies. Sera used for isolation of anti-Id antibodies from subjects 07, 08, and 23 were obtained at the time when all 3 showed the highest concentration of anti-mouse globulin antibodies (see Fig. 1). Patient 08 received a second injection of 130 mg of mAb 20 months after the first injection, and serum obtained after this *second* injection was used in a screening test for the presence of anti-Id (Table 1).

Preparation of Rabbit Anti-Idiotypic Antibodies. New Zealand White rabbits were injected subcutaneously at multiple sites with 300 μg of purified mAb 17-1A emulsified in Freund's complete adjuvant and, 30 days later, were injected intramuscularly with 100 μg of mAb 17-1A. Sera were col-

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Abbreviations: anti-Id, anti-idiotype; mAb, monoclonal antibody; CRC, colorectal carcinoma.

lected on day 10 of the secondary response. Antisera were adsorbed on mAb $C_4$2032 and on mAb 17-1A immunoadsorbents as described below.

Isolation of Anti-Idiotypic Antibodies from Human Sera. Immunoadsorbents were prepared by coupling purified mAbs (30 mg each) to 2 ml of Affi-Gel 10 (Bio-Rad). Sera were obtained from patients injected with one dose of mAb 17-1A (10, 14). Serum samples previously shown by radioimmunoassay to contain anti-murine IgG antibody were sequentially adsorbed on mAb $C_4$2032 and 17-1A immunoadsorbents to remove anti-isotypic and anti-idiotypic antibodies, respectively. Adsorbed antibodies were eluted with 0.1 M glycine buffer (pH 2.8), immediately neutralized with phosphate buffer, and dialyzed against phosphate-buffered saline; the protein was quantitated by absorption at 280 nm ($A_{280}^{1\%} = 14$).

Competition Assay for Detection of Anti-Id. Polystyrene beads (0.25 in, 6.35 mm) (Precision Plastic Ball, Chicago; Lot C-2084) were washed three times with 95% ethanol. The air-dried beads were incubated overnight at 4°C with gentle shaking with a dilution of either rabbit or human anti-Id antibody in 0.02 M sodium tetraborate (pH 8.2), then washed three times with phosphate-buffered saline and further incubated for at least 3 hr at room temperature with phosphate-buffered saline/2% bovine serum albumin/0.04% NaN_3. The beads were then exposed to ^{125}I-labeled mAb 17-1A as the reference idiotype and incubated with the potential source of human anti-Id—i.e., human sera diluted to 25% concentration in Ca^{2+}-, Mg^{2+}-free phosphate-buffered saline/2% bovine serum albumin/0.04% NaN_3. After an additional overnight incubation, the beads were washed and the radioactivity bound was measured in a gamma counter.

Stimulation of Peripheral Blood Mononuclear Cells. Peripheral blood mononuclear cells were stimulated with $F(ab')_2$ fragments at 10 ng/ml of mAb 17-1A *in vitro* as described (15) to activate antigen-specific T cells. During the following 7 days, aliquots of cells were separated into T- and B-cell populations by rosetting with sheep erythrocytes treated with 2-aminoethylisouronium bromide (16). Both populations were stained with $F(ab')_2$ fragments of mAb 17-1A or anti-influenza mAb and goat anti-mouse fluorescein-conjugated Ig and subsequently analyzed in a cytofluorograph. In addition, peripheral blood mononuclear cells from the same patient were stimulated with $F(ab')_2$ fragments of mAb 17-1A or anti-influenza mAb for 9 days in a modified Mishell–Dutton culture for specific human Ig production (15). Supernatants from these cultures were assayed in a solid-phase enzyme-linked immunoabsorbent assay for specific human IgG (KPL Laboratories, Gaithersburg, MD).

RESULTS

To screen serum samples for the presence of anti-Id, a competition assay was performed using rabbit anti-Id and four human sera previously incubated with ^{125}I-labeled mAb 17-1A. The results (Table 1) indicate that the three sera (nos. 23, 09, and 14) obtained after one injection of mAb showed inhibition of binding of mAb 17-1A that was higher than that of pre-mAb injection samples. Binding inhibition values obtained for post-mAb injection serum of patient 14 were low as compared with those of the other two sera but higher than for the pre-mAb exposure serum of the same subject. Inhibition values for serum obtained from patient 08, 7 days after he received a *second* injection of mAb were already high. This crude rapid screening assay with polyclonal rabbit anti-Id serum was followed by isolation and purification of individual anti-Ids by the immunoadsorbent technique described in *Materials and Methods*. Anti-Ids isolated from serum of subject 23, who received 750 mg of mAb, and from sera of subjects 08 and 07, who received 133 and 125 mg of mAb, respectively (obtained after the first mAb injection at times indicated in Fig. 1), were shown to be human immunoglobulins by binding to ^{125}I-labeled anti-human $F(ab')_2$ fragments. The yield of anti-Id protein from serum samples varied: from no. 08, 13 μg/ml; from no. 07, 8.9 μg/ml; from no. 23, 43 μg/ml. The largest amount was obtained from no. 23,

Table 1. Inhibition of binding of ^{125}I-labeled mAb 17-1A to rabbit anti-Id 17-1A by patients' sera

Serum no.	Days after mAb injection	% inhibition of binding of ^{125}I-labeled 17-1A to rabbit anti-Id 17-1A		
		10%	25%	50%
23	0*	0	21	2
	108	25	96	73
	184	17	NT	87
09	0*	NT	20	NT
	28	NT	46	NT
14	0	0	NT	0
	49	10	NT	17
08	0*	0	21	NT
	7†	31	64	NT

Rabbit anti-Id 17-1A (7.5 μg/ml) was coupled to polystyrene beads. ^{125}I-labeled 17-1A (12,500 cpm) was first incubated on ice with 10%, 25%, or 50% serum for 1 hr and then added to the coupled beads. Percentage of inhibition of binding of ^{125}I-labeled 17-1A to rabbit anti-Id 17-1A was calculated by the following formula: % binding inhibition = 100 − [(100 × cpm bound in presence of serum)/cpm bound without serum]. NT, not tested.
*Sample obtained immediately before mAb injection.
†Serum obtained 7 days after second administration of mAb.

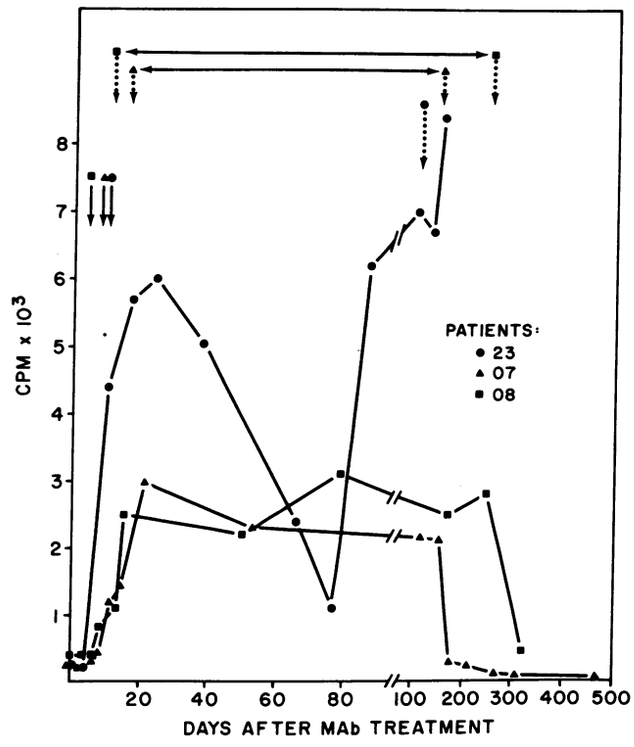

FIG. 1. Presence of human anti-mouse globulin antibody in sera of three subjects as determined by binding to mouse mAb 17-1A in the presence of ^{125}I-labeled rabbit anti-human $F(ab')_2$ immunoglobulin (14). Dotted arrows indicate days after treatment when serum samples used for isolation of anti-idiotypic antibody were taken (one sample from patient 23, pooled sera from patients 07 and 08). Solid arrows indicate the last day of circulation of mAb 17-1A in patient blood.

Table 2. Binding of human anti-idiotype 17-1A antibodies to monoclonal antibodies

Patient	Anti-Id, μg/ml	Binding of ^{125}I-labeled mAb to anti-Id, cpm			
		17-1A	$C_4$2032	$C_4$1472	A5C3
08	<5.0	1,693 ± 131	214 ± 31	308 ± 44	166 ± 18
07	6	1,023 ± 91	168 ± 26	363 ± 21	251 ± 29
23	18	21,339 ± 156	427 ± 24	512 ± 59	813 ± 101
	7	7,956 ± 293	208 ± 15	NT	427 ± 23

cpm bound to normal human IgG (40 μg/ml) of ^{125}I-labeled 17-1A, $C_4$2032, and A5C3 were 151 ± 25, 139 ± 32, and 247 ± 32, respectively. Results represent mean ± SD of triplicate determinations. NT, not tested.

which also showed the highest levels of anti-mouse globulin antibodies (Fig. 1).

Binding of the isolated anti-Id to mAb 17-1A and to three other mAbs is shown in Table 2. The results indicate that the binding of anti-Ids from all three sera to mAb 17-1A was significantly higher than to the three other mAbs, two of which ($C_4$2032 and $C_4$1472) also detect antigenic sites on CRC cells, though these sites are different from those recognized by mAb 17-1A (10). Immunoglobulin isolated from sera of all three subjects prior to exposure to mAb 17-1A was adjusted to approximately 2.5 μg/ml and coupled to polystyrene beads. These preparations did not bind any of the mAbs listed in Table 1, indicating the absence of anti-Id in pre-exposure serum.

These results indicate that anti-Id could be detected by the screening assay in four sera of patients injected with mouse mAb and that anti-Id could be isolated and purified from three sera reacting only with the idiotype injected into the patient.

Crossreactivity Between Human Anti-Ids. The results of the competition assay (Table 3) indicate significant crossreactivity between the anti-Ids of sera of patients 07 and 23 and slightly less but still significant crossreactivity between anti-Id sera of patients 08 and 23. Similar crossreactivity was found between anti-Id 07 and post-mAb serum obtained from no. 08 (results not shown). These results suggest that the anti-Ids in different patients are directed against the same site.

Is the Anti-Id Directed to the Framework or Antigenic Site of the Idiotype Determinants? To answer this question, ^{125}I-labeled mAb 17-1A was incubated with a 3 M KCl cell extract of a human colon carcinoma that binds mAb 17-1A and with 3 M KCl extract of melanoma cells that does not bind mAb 17-1A. The antibody–antigen mixture was then added to no. 23 anti-Id-coated beads and binding was compared with binding of the radiolabeled mAb alone. These experiments were performed with nonsaturating amounts of iodinated mAb to detect changes in binding with small amounts of competitive haptens. For control purposes, the iodinated mAb 17-1A was mixed with an extract from melanoma cells that had previously been shown to not bind mAb 17-1A. The CRC cell extracts in concentrations of 0.1 or 0.5 mg/ml were found to inhibit the binding of anti-Id to iodinated mAb 17-1A by 39% and 68%, respectively, whereas the extract from melanoma cells in concentrations up to 0.5 mg of protein/ml did not significantly affect mAb binding (Table 4). This hapten inhibition of the binding reaction suggests the presence of an "internal image" of the CRC epitope on the anti-Id molecule, as supported by the finding that the 3 M KCl extract of CRC cells did not bind to the anti-Id but did bind, as expected, to mAb 17-1A.

Table 3. Crossreactivity between human anti-Ids in competition assay for detection of anti-Id

Antibody		cpm bound	% inhibition of mAb 17-1A binding
First	Second		
Anti-Id 23	None	4,297	
	07 Pre-mAb	4,595	0
	Post-mAb	1,231	71
	08 Pre-mAb	4,097	5
	Post-mAb	2,585	40

Stimulation of B Cells by mAb 17-1A. Buffy coat cells were obtained from patient 23, 5 months after injection of mAb, and from patient 08, 20 months after injection of mAb. The percentage of lymphocytes that specifically bound 17-1A F(ab')$_2$ of patient 08 was 1.2 and that of patient 23 was 0.2. During 7 days in culture with mAb 17-1A, the percentage of lymphocytes of patient 23 that specifically bound 17-1A F(ab')$_2$ increased from 0.2 to 13. All the 17-1A-binding cells were present in the B-cell population. In addition, after 9 days, human anti-17-1A mAb IgG was detected. Incubation of lymphocytes from the same patient with anti-influenza mAb under identical conditions produced no detectable human Ig to either mAb 17-1A or anti-influenza mAb.

Patients Who Responded with a Remission After mAb Treatment. Table 5 gives data on five patients, four of whom had a small to medium tumor burden of metastatic or recurrent colorectal adenocarcinoma (14) and one, no. 14, who had a pancreatic carcinoma with small liver metastases. The tumors in all five patients regressed after a single administration of mAb 17-1A. Anti-Id was detected in the sera of four patients in the screening test, and the anti-Id was isolated and purified from sera of three other patients. Three patients (nos. 07, 09, and 14) are currently without evidence of tumor growth. Patient 08 had a local recurrence of tumor 18 months after mAb 17-1A treatment without any additional therapeutic measures. It is too soon to evaluate the long-term therapeutic results in patient 23.

DISCUSSION

mAb 17-1A has been used successfully to diagnose gastrointestinal cancers in humans by binding to tumor tissue *in situ*

Table 4. Hapten inhibition of binding of human anti-Id to mAb 17-1A

Hapten		% inhibition of binding of anti-Id 23 to ^{125}I-labeled 17-1A
Source	Conc., mg/ml	
CRC SW-1222	0.5	68.5
	0.1	39.4
MEL SK-MEL-21	0.5	6.6
	0.1	0

CRC = colorectal cancer tissue culture line. MEL = melanoma tissue culture line. ^{125}I-Labeled mAb 17-1A was mixed with 3 M KCl cell extracts to final extract concentrations of 0.5 and 0.10 mg/ml and 62,500 cpm of iodinated mAb per ml. After a 1-hr incubation on ice, 200 μl of the mixture or 200 μl of iodinated mAb alone was added per polystyrene bead coupled with anti-Id 17-1A (15 μg/ml; patient 23) and this mixture was incubated overnight at 4°C. Results were calculated by the following formula: % inhibition of binding = 100 − [(100 × cpm bound in the presence of extract)/cpm bound without extract].

Table 5. Anti-Id response in patients who had a disease remission after one injection of mAb 17-1A

Patient	17-1A mAb treatment		Anti-Id response	
	Date	Amount, mg	Screening*	Isolation†
07	December 1981	125	ND	+
08	December 1981‡	92	+	+
09	March 1982	133	+	ND
14	September 1982	433	+	ND
23	February 1983	845	+	+

ND, not done.
*See Table 1.
†See Table 2.
‡Remission lasted 18 months.

and by detection of the radiolabeled antibody by gamma scintigraphy (10). This does not preclude the possibility that the tumor antigen defined by mAb 17-1A may act as an immunogen to elicit host immune mechanisms for the control of tumor growth. As several patients benefited from treatment with mAb 17-1A, a functional involvement of immune mechanisms specifically connected with the 17-1A-defined antigen is suggested.

Although mAb may directly destroy tumor cells by activation of complement or by interaction with macrophages or killer cells (17, 18), it is possible that other mechanisms may be operative in the present study because of the need for very large amounts of mAb to interact with all antigenic sites of target cells of a solid tumor mass and because of the long induction period to measure the antitumor effect. In fact, the decrease in size and eventual disappearance of lung metastases, which could be measured by radiography, occurred over 3–4 months in one patient (10). Thus, although one may expect to observe an antitumor effect a long time after the administration of mAb, binding of mAb to metastatic tumor biopsy samples was detected only up to 1 wk after injection of mAb (results not shown).

An alternative mechanism might involve a network of interacting anti-idiotypic T and B cells directed against the mAb. Among them may be those that react with the mAb binding site. Such anti-Id would be considered internal images of the tumor antigen. The hapten inhibition binding described in the present paper suggests that an internal image of the 17-1A CRC antigen was produced by the anti-Id response. Although it is still difficult to correlate the presence of anti-Id with the outcome of the immunotherapy, it should be mentioned that three patients who produced anti-Id have had tumor regression and currently have no detectable disease. One had remission lasting 18 months, and it is still too early to evaluate the outcome in the fifth patient. Sera of three other patients who are apparently in remission were studied for the presence of anti-Id but they did not seem to develop anti-mouse globulin antibodies. The assay for the latter does not reflect the immune status of the individual since B cells of one of the patients are stimulated in vitro by mAb 17-1A similarly to patients who had had circulating anti-mouse globulin antibodies (results not shown). In such cases repeated injection of mouse mAb could disturb the preexisting immune state, boosting the production of anti-Id that may or may not be reactive with other determinants on mouse immunoglobulin. Because the test for detection of antitumor antibody in cancer patients is rather insensitive, the determination of an anti-Id response, which detects small amounts (<5.0 μg) of the antibody, may be the only indication of functional changes in the network system.

As all human anti-Ids showed crossreactivity, it seems likely that the inoculation of mAb 17-1A in other subjects could induce production of crossreacting anti-Ids and possibly identical internal images of the cancer antigen. The presence of an internal image of an antigen on a human immunoglobulin molecule, in contrast to the presence of the antigen on a tumor cell, may change the conditions under which the immune system reacts to the tumor antigen. Modulation of the immune response of the subject as a result of such antigen presentation may explain a successful outcome of immunotherapy.

The most direct evidence for a beneficial effect of anti-Id would be the administration of anti-Id produced in a patient to a nonresponsive cancer patient and the triggering of an antitumor response, which could be measured *in vivo* and *in vitro*. This may not be feasible because of the paucity of human anti-Id. One must turn, therefore, to the production of xenogeneic anti-Id, which can be obtained in animals without difficulty. The slight advantage of this approach lies in the possibility of using a variety of mAbs to produce anti-Id antibodies and, if internal images are produced, they may "mimic" a large number of heterogeneous antigens of the tumor cells.

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