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Anti-murine Antibody Response to Mouse Monoclonal Antibodies in Cancer Patients

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Although development of human anti-murine immunoglobulin antibody (HAMA) is often seen in patients receiving murine antibodies, the variety of methods used for detecting HAMA makes it difficult to compare directly the HAMA responses measured by different assays. In the present study, several parameters of the HAMA response to two murine monoclonal antibodies were evaluated. The anti-sialosyl Tn antibody MLS102 and anti-CA125 antibody 145-9, which were labeled with ^{111}In, were injected intravenously into 17 colorectal cancer patients and 11 ovarian cancer patients for immunoscintigraphy, respectively. HAMA was measured by enzyme-linked immunosorbent assay. There was no difference in baseline HAMA levels before antibody injection between the two groups. HAMA developed more frequently in ovarian cancer patients receiving the 145-9 antibody than in colorectal cancer patients receiving the MLS102 antibody (9/11 vs. 6/17, $P<0.05$). No significant difference was observed in maximal HAMA levels between the two groups of patients. However, time to reach the maximal levels was delayed and the duration of the response seemed longer in ovarian cancer patients. Among 11 patients receiving the 145-9 antibody three patients became positive for HAMA more than 2 months after antibody injection and the other two had HAMA activity in their sera for more than 17 months. HAMA response was different between the two antibodies, and late onset or long duration of HAMA response against the 145-9 antibody suggests the importance of HAMA measurement in patients who receive a second injection of murine antibodies even after a long interval.

Key words: Anti-mouse IgG antibody — Murine monoclonal antibody — Ovarian cancer — Colorectal cancer

Monoclonal antibodies have been used for immunoscintigraphy and targeted therapy of cancer. Most of the antibodies currently in clinical use are of murine origin. Development of human anti-murine immunoglobulin antibody (HAMA) is often seen in patients receiving murine antibodies.[1–6] When murine antibodies are administered again to patients who have had HAMA, the injected antibodies form complexes with HAMA and are rapidly cleared from the circulation.[7] As a result, effective targeting is not achieved.

There are many variables which influence the development of HAMA, such as antibody dose, use of whole IgG or fragments, immunocompetence of patients, etc. Although the incidence of HAMA may vary among different antibodies, the variety of methods and techniques for detecting HAMA makes it difficult to compare directly the HAMA responses to each antibody measured by different assays. Many studies on the HAMA response have been reported. However, only a few reports have dealt with Japanese patients. The immune response against murine antibodies could be different among races. In fact the incidence of HAMA against an anti-myosin antibody in Japanese patients was higher than that found in an American multicenter clinical trial.[8, 9]

We have employed two immunoscintigraphy protocols using two different murine antibodies to assess several parameters of the HAMA response in two distinct groups of Japanese patients. There was a difference in the incidence and peak time of response between the two groups.

MATERIALS AND METHODS

Antibodies MLS102[10] is a murine monoclonal IgG_3 antibody which recognizes sialosyl Tn on mucin. The antibody was purified from ascitic fluid of hybridoma-bearing mice. The murine monoclonal antibody 145-9[11] (IgG_{2b}) which reacts with CA125 was purified from the hybridoma culture supernatant. The antibodies were labeled with ^{111}In using diethylenetriaminepentaacetic acid as a chelate. The procedures used for purification and labeling of the antibodies were reported previously.[12, 13]

Patients Seventeen patients with colorectal cancer received the MLS102 antibody and 11 patients with ovarian cancer received the 145-9 antibody according to the immunoscintigraphy study protocol described previously.[12, 13] Two milligram aliquots of the ^{111}In-labeled antibodies were injected intravenously. None of the patients had received murine antibodies before. All patients gave their informed consent to participation in the study, which was approved by the Ethical Review Committee of the Faculty of Medicine, Kyoto University.

One patient with colorectal cancer received tegafur daily p.o. before and after antibody injection. Ten patients with ovarian cancer received intensive chemotherapy including cisplatin within 1 month before antibody injection or within 2 weeks after antibody injection (Table I).

Measurement of HAMA HAMA was measured using an enzyme-linked immunosorbent assay (ImmuSTRIP HAMA IgG; Immunomedics Inc., Warren, NJ). The HAMA assay was a 2-step test carried out in plastic microwell strips coated with whole mouse IgG. The HAMA was then sandwiched between solid-phase mouse IgG and mouse IgG conjugated with horseradish peroxidase. The test was standardized against primate anti-mouse IgG serum and values are reported as nanograms of precipitable antibody equivalents per milliliter. To characterize HAMA in the patients, neutralization tests were performed. These were done by adding 50 μg/50 μl of mouse IgG (Sigma Chemical Co., St. Louis, MO) to the assay of nine HAMA-positive samples from 2 colorectal and 6 ovarian cancer patients, and by adding 50 μg/50 μl of mouse Fab fragment of IgG (Rockland, Gibertsville, PA) to the assay of ten HAMA-positive samples obtained from 2 colorectal and 8 ovarian cancer patients.

Blood samples were taken prior to the antibody injection and at 1, 3–4 weeks or more after administration. The follow-up period for HAMA was between 31 days and 22 months (mean: 13.6 months) in patients receiving the MLS102 antibody and between 30 days and 24 months (mean: 12.2 months) in patients receiving the 145-9 antibody.

Table I. Parameters of HAMA Response

	Age (years)	Sex	Chemotherapy pre-/post-antibody injection	Baseline HAMA (ng/ml)	Maximal HAMA (ng/ml)	Onset	Peak time	Last day when HAMA >62.5 ng/ml	Duration	Follow-up period
Colorectal cancer patients receiving MLS102 antibody										
1	58	M	—	4.4	23.8	—	—	—	—	12M
2	68	M	—	0.0	30.9	—	—	—	—	12M
3	64	M	—	0.0	67.5	30D	30D	30D	NA	21M
4	60	F	pre, post	0.0	15.4	—	—	—	—	3M
5	58	M	—	0.0	20.3	—	—	—	—	19M
6	67	F	—	0.0	25.0	—	—	—	—	18M
7	59	M	—	11.7	82.1	28D	28D	28D	NA	19M
8	71	F	—	0.0	14.5	—	—	—	—	15M
9	67	M	—	0.0	4041.0	7D	28D	5M	>5M	5M
10	78	F	—	21.3	21.3	—	—	—	—	15M
11	61	F	—	13.8	41.3	—	—	—	—	15M
12	41	F	—	19.4	694.4	23D	23D	10M	9M	24M
13	71	F	—	13.2	585.3	28D	28D	4M	3M	22M
14	43	M	—	6.9	12.4	—	—	—	—	22M
15	60	M	—	1.9	11.8	—	—	—	—	5M
16	37	M	—	88.6	6160.0	20D	20D	3M	>2M	3M
17	84	F	—	22.9	60.6	—	—	—	—	31D
Ovarian cancer patients receiving 145–9 antibody										
1	60	F	—	20.0	24.0	—	—	—	—	24M
2	40	F	post	11.3	778.0	7D	8M	14M	14M	22M
3	52	F	post	19.1	11040.0	7D	7M	22M	>22M	22M
4	62	F	post	17.0	3775.0	23D	8M	18M	>17M	18M
5	43	F	post	12.4	68.2	2M	2M	2M	NA	12M
6	62	F	pre	8.2	107.9	28D	28D	28D	NA	3M
7	64	F	post	3.1	872.0	11M	11M	11M	NA	11M
8	26	F	post	8.3	2185.0	13D	13D	9M	>9M	9M
9	44	F	post	0.0	1.6	—	—	—	—	8M
10	36	F	post	0.0	2703.0	4M	4M	4M	NA	4M
11	72	F	post	0.0	1153.6	30D	30D	30D	NA	30D

D, days after antibody injection; M, months after antibody injection; NA, not applicable.

Table II. HAMA Responses to Two Antibodies

	Baseline HAMA levels (ng/ml)	Positive rate	Maximal HAMA levels[a] (ng/ml)	Onset[a] (month)	Peak time[a] (month)	Last day when HAMA>62.5 ng/ml[a] (month)
MLS102	12.6±21.3	6/17	1938±2252	0.8±0.3	0.9±0.1	4.0±3.4
145-9	9.0±7.6	9/11	2520±3423	2.3±3.5	4.7±3.9	9.1±7.8
Statistical significance	ns	$P<0.05$	ns	ns	$P<0.05$	$P<0.05$

a) These parameters were calculated for the 6 colorectal cancer patients and 11 ovarian cancer patients who showed maximal HAMA levels over 62.5 ng/ml.

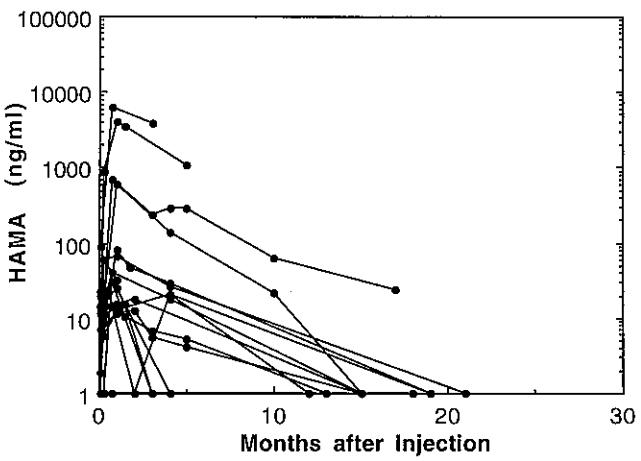

Fig. 1. HAMA in patients receiving MLS102 antibody.

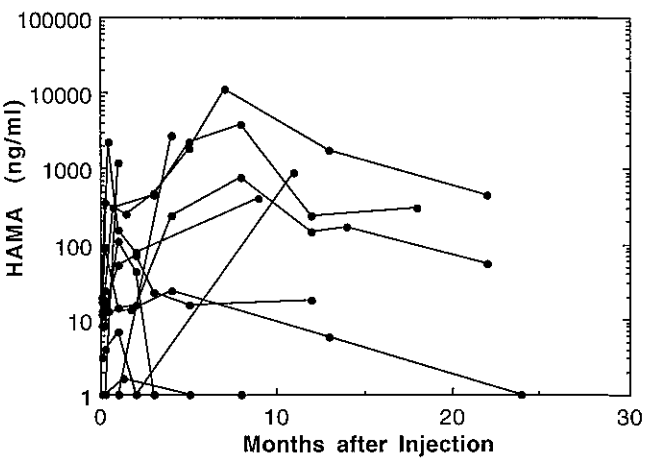

Fig. 2. HAMA in patients receiving 145-9 antibody.

Statistical analysis All data expressed as the mean±SD. The χ^2 test was used to compare the incidences of HAMA response. Other parameters of the response were compared using the unpaired t test. A probability value of <0.05 was considered significant.

RESULTS

The HAMA titer of baseline serum obtained before antibody injection from all patients was 11.2±17.1 ng/ml and there was no significant difference between the two groups (Tables I, II). Values above 62.5 ng/ml (mean±3SD) were considered positive.

Time courses of HAMA titer are shown in Figs. 1 and 2. Several parameters of the response are summarized in Table II. HAMA developed more frequently in ovarian cancer patients receiving the 145-9 antibody than in colorectal cancer patients receiving the MLS102 antibody. Six of the 17 patients (35%) with colorectal cancer became positive for HAMA, while HAMA developed in 9 of the 11 patients (82%) with ovarian cancer. No significant difference was observed in maximal HAMA levels between the two groups of patients. The onset of the response seemed later and time to reach the maximal HAMA level was delayed in ovarian cancer patients receiving the 145-9 antibody. The duration of the response also seemed longer in ovarian cancer patients, although the follow-up period in colorectal cancer patients with a HAMA titer over 1000 ng/ml was less than 5 months. Maximal HAMA levels were reached within 1 month and then decreased in all six positive patients with colorectal cancer. On the other hand, of the nine positive patients with ovarian cancer, six showed maximal HAMA levels more than 2 months after injection of the 145-9 antibody. In one patient, a high level of HAMA was first detected at 11 months.

Although the incidence of HAMA was higher in ovarian cancer patients, there was no difference in parameters of HAMA response depending on gender in colorectal cancer patients. No parameter of HAMA response correlated with age of patients.

After the addition of mouse IgG to the HAMA assay of HAMA-positive sera, the absorbance decreased to less than 7% (data not shown), which suggested that the assay accurately detected antibodies recognizing the constant region of mouse IgG, and unknown antigens did

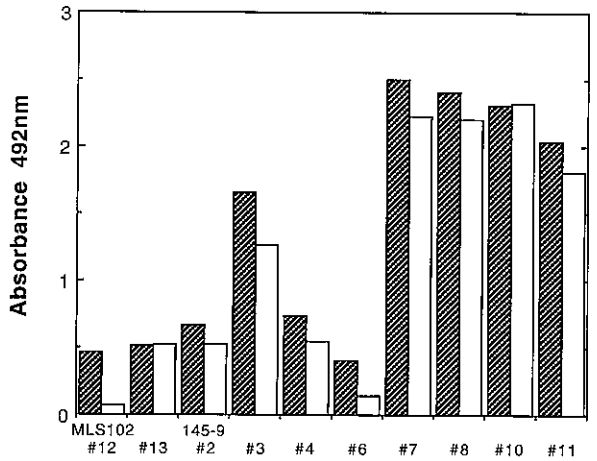

Fig. 3. Neutralization test. Decrease of absorbance when Fab fragment was added to the HAMA assay indicates the presence of HAMA recognizing the CH1 and/or CL region of mouse IgG. ▨ control, □ addition of Fab fragment to the assay.

not interfere with the assay. The addition of mouse Fab fragment decreased HAMA activity to various extents (Fig. 3), indicating that HAMA in the patients' sera recognized not only the Fc portion, but also the CH1 and/or CL domains of mouse IgG.

DISCUSSION

Although the assay used in the present study did not detect anti-idiotype antibodies, HAMA after a single injection of mouse antibody is usually cross-reactive with other murine immunoglobulin G.[1] Therefore, most of the HAMA was supposed to be detected by this assay, even if the patients had low levels of anti-idiotype antibody. We could easily compare HAMA responses in the two groups of patients with the same assay by neglecting anti-idiotype antibodies.

In previous studies, we selected 400 ng/ml as a cutoff value according to the literature.[14] As the HAMA titer did increase after the antibody infusion in patients whose titer did not reach 400 ng/ml, in the present study we set the cutoff value at 62.5 ng/ml from the baseline titer.

The positive rate of HAMA was higher and the duration of the response was longer in patients receiving 145-9. There were no differences between the two antibody imaging regimens with regard to the injected dose, antibody form, or radiolabeling procedure. Most of the patients had pre-existing HAMA before antibody injection, although the titer was very low. There are many reports in the literature concerning the presence of pre-treatment anti-mouse antibodies[5] and Schroff *et al.*[1] suggested that differences in the HAMA response may be related to pre-existing antiglobulin level. In the present study, however, baseline HAMA levels were not different between the two groups and we could not find any correlation between baseline HAMA levels and subsequent elevation of HAMA titer. Differences in HAMA production may be due to the difference in isotype or some unknown parameter of the molecular structure of antibodies, although a previous study suggested that the induction of HAMA was not related to the isotype of the administered antibody.[4]

Another difference was the level of circulating antigens. A large amount of 145-9 antibody formed complexes with antigens in the circulation, which survived for several days.[13] In contrast, MLS102 did not form such high levels of immune complexes, and complexes which were formed disappeared rapidly (data not shown). It is suggested that complex formation between the injected murine antibody and circulating antigens enhances the immunogenicity of the antibody.[15] The antibody OC125 also forms complexes with circulating CA125 *in vivo*[16,17] and both the OC125 antibody and another antibody specific for CA125 are highly immunogenic.[18,19] These findings suggest that high immunogenicity is a common property among antibodies recognizing CA125. Circulating antigen and immune complex formation may have some role in the production of HAMA.

The ovarian cancer patients would have been rather immunosuppressed because of intensive chemotherapy. However, it is not likely that immunosuppressed patients would develop HAMA more frequently. Shawler *et al.*[2] were unable to correlate the lack of response to any of a large number of clinical parameters, and it still remains difficult to predict from the clinical data which patients will develop antibodies. The tendency for delayed HAMA response in ovarian cancer patients may be related to immunosuppression. When patients are given intravenous infusions of whole murine IgG, there is usually a rapid development of immunity, although the first detection of HAMA is sometimes very late.[20] It should be noted that patients may become positive for HAMA several months after injection of some murine antibodies. Long duration or late onset of the response suggests that measurement of HAMA is important in patients who receive a second injection of murine antibody even after a long interval. Positive results in such a measurement can prevent the ineffective administration of antibody for tumor targeting.

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