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Development of Human Antimouse Antibodies (HAMA) After Single and Repeated Diagnostic Application of Intact Murine Monoclonal Antibodies

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ABSTRACT

The HAMA response after diagnostic application of murine monoclonal antibodies was examined in 67 patients. In 23 cases HAMA's were determined before and three months after administration of 1 mg intact monoclonal anti-CEA antibody (MAb BW 431/26), in 29 patients before and three months after application of 0.5 mg intact monoclonal antigranulocyte antibody (MAb BW 250/183). In another group of 15 patients, HAMA's were determined at three-week intervals before and after repeated application of 0.3 mg MAb BW 250/183. In all patients total HAMA response (IgM and IgG) as well as the proportion of antiidiotypic response (IgM and IgG) was determined by an enzyme immuno assay (Enzygnost HAMA micro, Behring, Marburg FRG). After single application of 1 mg MAb BW 431/26, 7 out of 23 patients developed HAMA's (6 patients with predominantly antiisotypic, 1 patient with predominantly antiidiotypic response). After administration of 0.5 mg MAb BW 250/183, 4 out of 29 patients developed HAMA's (3 patients with predominantly antiisotypic, 1 patient with predominantly antiidiotypic response). At repeated application of 0.3 mg MAb BW 250/183 none of the 15 patients developed human antimouse antibodies after the first application; after the second and third application one patient showed a clear increasing antiidiotypic response one patient a marginal antiidiotypic response, two patients an antiisotypic response and 4 patients a transient predominantly antiisotypic IgM response. Deterioration of image quality in repeated immunoscintigraphic investigations was observed in cases of high HAMA titers and/or predominantly antiidiotypic HAMA response.

INTRODUCTION

With the introduction of Tc-99m-labeled monoclonal mouse antibodies, immunoscintigraphy has become a routine method at a number of centres, especially in the diagnosis and follow-up of colorectal carcinomas and the identification of inflammatory processes. Its employment has been encouraged by the ready availability of Tc-99m as well as its high sensitivity and specificity (1,2,3,4,5). However, the utility of a method depends, inter alia, on whether it can be applied repeatedly also in the follow-up of diseases (e.g. colorectal recurrence, lymph node and distant metastases; Crohn's disease, ulcerative colitis, osteomyelitis). The formation of human antimouse antibodies (HAMA) could considerably reduce the potential of immunoscintigraphy for follow-up because of the deterioration of image quality. Literature provides greatly differing and sometimes confusing information on the actual frequency of HAMA response and on the consequences for repeated examinations. The results usually refer to therapeutic applications of monoclonal mouse antibodies, with dosages varying from 6 to 400 mg and 100 to 700 mg respectively in the groups investigated (6,7). As far as the results of diagnostic application of monoclonal mouse antibodies are concerned, data on the doses of antibodies administered is partly not available or HAMA response data of different monoclonal antibodies were subsumed (8,9).

Thus it has been the aim of our study to investigate the frequency of HAMA response at defined diagnostic antibody administration after single and repeated applications.

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PATIENTS AND METHODS

In 67 patients the total HAMA response as well as the percentage of antiisotypic and antiidiotypic response after diagnostic immunoscintigraphy were determined.

Group I: HAMA response after 1 mg MAb BW 431/26

In 23 patients (15 females, 8 males; age 61 ± 11 years) with suspicious primary colorectal carcinomas or colorectal recurrences the HAMA values could be determined before and three months after application of 1 mg antibody (MAb BW 431/26, Behring Werke, Marburg, FRG).

Group II: HAMA response after 0.5 mg MAb BW 250/183

In 29 patients (16 females, 13 males; age 50 ± 9 years) the HAMA values were determined before and three months after application of 0.5 mg antibody (MAb BW 250/183; Behring Werke Marburg, FRG).

Group III: HAMA response after repeated application of 0.3 mg MAb BW 250/183

In 15 patients (11 males, 4 females; age 42 ± 12 years) with complicated bone fractures HAMA values were determined before and three weeks after first application, six weeks after first application and before second application respectively, three weeks after second application, six weeks after second application and before third application respectively, and three weeks after third application of 0.3 mg MAb BW 250/183. In addition, the liver uptake 18 hours post injection - liver activity/total injected activity - corrected for decay and background was determined during the first and third immunoscintigraphy in group III.

Indirect enzyme immunoassay for HAMA determination

Monoclonal antibodies (unspecific antibody; BW 431/26 = specific anti-CEA antibody; BW 250/183 = specific anti-granulocyte antibody) were diluted to 1 ug/ml in the dilution medium and applied onto microtiter plates (Enzygnost HAMA micro; Behring Werke Marburg FRG) coated with goat polyclonal antibodies to mouse IgG. The plates were incubated at 37° C for one hour and then washed three times with 250 ul of diluted washing solution (phosphate buffer solution containing 0.05%tween). 100 ul of the dilution medium, diluted negative control, positive control and patient serum (1:10) were given into the wells, and the plates were incubated at 37°C with 100 ul of a 1:50 diluted IgG/POD (rabbit antihuman immunoglobulin/peroxidase) or IgM/POD (goat antihuman immunoglobulin/peroxidase) conjugate. The plates were then washed again three times, and 100 ul of the TMB (3,3',5,5'tetramethylbenzidine) working solution were given into the wells and incubated, protected from light, at +20°C for 30 minutes. After addition of 100 ul of stopping solution (o.5 M sulfuric acid), the absorbance was determined at 436 nm. After reading the absorbance values, the HAMA factor was determined as follows: HAMA factor = serum samples - blank/ negative control - blank. For normal values the HAMA factor against unspecific monoclonal mouse antibodies and specific monoclonal antibody (BW 431/26, BW 250/183) were calculated in 50 (BW 431/26) and 34 (BW 250/183) healthy subjects respectively (Table 1,2).

Baseline HAMA values (before immunoscintigraphy) were considered positive when their HAMA factor was greater than the mean + 2s of the HAMA factor from the 50 and 34 healthy subjects respectively. HAMA response values after immunoscintigraphy were considered positive when HAMA factor was twofold higher than the baseline value. To differentiate between antiisotypic and antiidiotypic HAMA response, the microtiter plates were incubated with the unspecific (antiisotypic

Table 1

Normal values of total and antiisotypic HAMA factor against MAb BW 431/26 in 50 healthy subjects

	x	s	range
IgM (total)	2.42	1.10	0.22-4.62
IgG (total)	1.65	0.52	0.61-2.69
IgM (iso)	2.40	1.18	0.04-4.76
IgG (iso)	1.55	0.44	0.67-2.43

HAMA: Human anti mouse antibody; total: antiiso- and antiidiotypic; iso: antiisotypic
x = average; s = standard deviation

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Table 2
Normal values of total and antiisotypic HAMA factor against MAb BW 250/183 in 34 healthy subjects

	x	s	range
IgM (total)	3.51	1.68	0.15-6.87
IgG (total)	2.05	0.86	0.33-3.97
IgM (iso)	2.39	1.02	0.35-4.43
IgG (iso)	1.32	0.57	0.18-2.50

HAMA: Human anti mouse antibody; total: antiiso- and antiidiotypic; iso: antiisotypic
x = average; s = standard deviation

response) and the specific (antiisotypic and antiidiotypic response) monoclonal antibodies. Antiidiotypic HAMA response was calculated as percentage of the total HAMA response minus antiisotypic HAMA response. This calculated antiisotypic HAMA response was compared with the results after inhibition. In this experiment, inhibition of HAMA in the patients sera was performed with the specific antibody (total inhibition) and the unspecific antibody (isotypic inhibition) before the first incubation. The percentage of antiidiotypic HAMA response was calculated as total inhibition minus antiisotypic inhibition/total inhibition.

RESULTS

Group I: Three months after the application of 1 mg of MAb BW 431/26, 7 of the 23 patients developed human antimouse antibodies.
In 6 cases the HAMA response (IgG) was primarily antiisotypic and in one case predominantly antiidiotypic (Table 3). In all 7 patients the IgM response was negative.

Table 3
HAMA factors (IgG) and percentage of antiiso- and antiidiotypic response 3 month after 1 mg MAb BW 431/26:

	A		B		C	
pts	HAMA factors		% response I		% response II	
	total	iso	iso	idio	iso	idio
1	5.93	3.68	62	38	83	17
2	5.96	5.73	96	4	84	16
3	8.64	4.92	56	44	58	42
4	8.70	7.25	83	17	78	22
5	13.76	11.14	81	19	83	17
6	16.11	4.65	29	71	25	75
7	25.58	22.81	98	11	81	19

Total and antiisotypic HAMA factors (A); calculated percentage of antiiso-and antiidiotypic response (B: % response I) and percentage of antiiso- and antiidiotypic response after total and isotypic inhibiton (C: % response II) in 7 patients with positive HAMA response.

Fig. 1. shows the whole body distribution (5 1/2 hours post injection) of the antibody before and after development of predominantly antiidiotypic HAMA in a patient with inoperable recurrence after left hemicolektomia.
Also in patients with high total HAMA factor and predominantly antiisotypic response the image quality is altered due to the high liver activity, but the demonstration of the lesions are still possible (Fig. 2, a, b).

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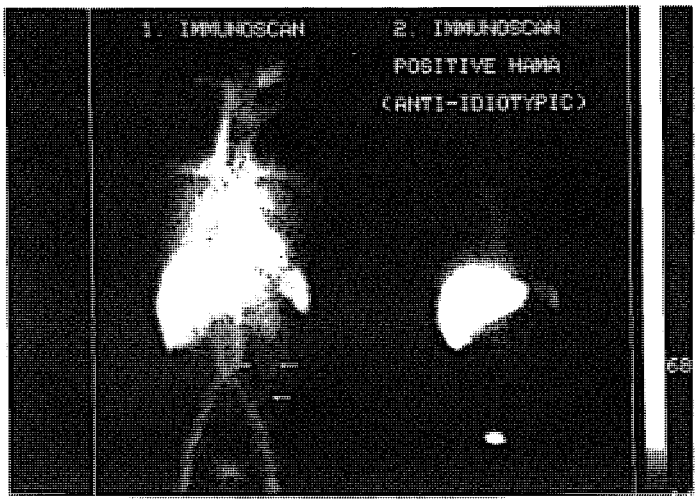

FIGURE 1: Whole body distribution of Tc-99m MAb BW 431/26 5 1/2 hours post injection in a patient with malignant recurrence after left hemicolektomia before (left side) and after (right side) development of predominantly antiidiotypic antibodies is shown. Whereas in the left image the malignant (inoperable) recurrence is clear demonstrated (arrow), the control scan after development of predominantly antiidiotypic HAMA's failed to image the recurrence.

For the percentage of antiidiotypic response there was a good correlation between the values calculated directly from the total and antiisotypic response and the values determined after total and antiisotypic inhibition.
Group II: Three months after application of 0.5 mg MAb BW 250/183, 4 of 29 patients examined showed human antimouse antibodies. In 3 cases the HAMA response was predominantly antiisotypic, in one case 50% antiisotypic and 50% antiidiotypic. Like in group I, there was a good correlation between the values determined directly and after inhibition (Table 4).

Table 4
HAMA factors (IgG) and percentage of antiiso- and antiidiotypic response
3 month after 0.5 mg MAb BW 250/183

	A		B		C	
pts	HAMA factors		% response I		% response II	
	total	iso	total	idio	total	iso
1	6.00	4.30	71	29	63	27
2	9.73	4.18	43	57	49	51
3	17.30	11.98	70	30	62	38
4	18.74	18.71	99	1	96	4

Total and antiisotypic HAMA factors (A); calculated percentage of antiiso-and antiidiotypic response (B: % response I) and percentage of antiiso- and antiidiotypic response after total and isotypic inhibiton (C: % response II) in 4 patients with positive HAMA response.

Group III: In a group of 15 patients examined after triple application (0.3 mg MAb BW 250/183), one patient developed increasingly antiidiotypic IgG human antimouse antibodies after the second and third application. The liver uptake rose from 3% to 24% in this patient (Fig. 3a, b; Tab.5).
In a second patient the HAMA value was just slightly elevated after second application, in two patients the IgG response was predominantly antiisotypic; in 4 patients there was only a transient predominantly antiisotypic IgM response. The remaining seven patients of group III developed neither transient nor permanent HAMA's up to three weeks after third application (15 weeks after first application). In these patients the liver uptake (basal values between 2% and 4%) did not show any significant increase after repeated immunoscintigraphy.

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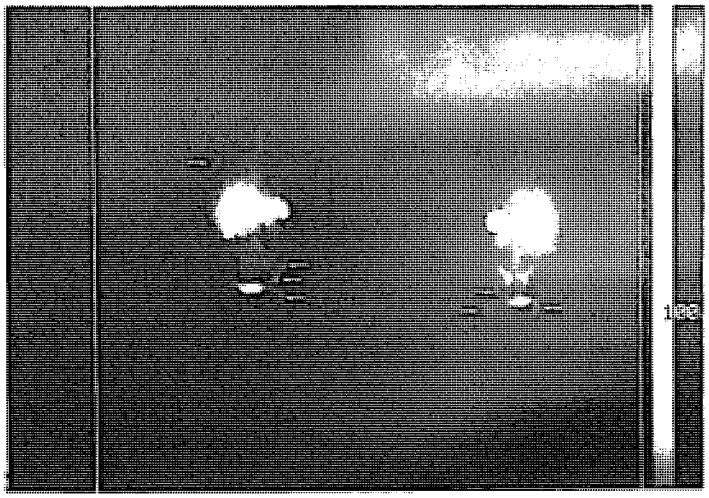

FIGURE 2a: Whole body scan of Tc-99m MAb BW 431/26 5 1/2 h post injection after development of predominantly antiisotypic HAMA's. The bone metastases in the right humerus, the left femur and the pelvis are still shown on the image.

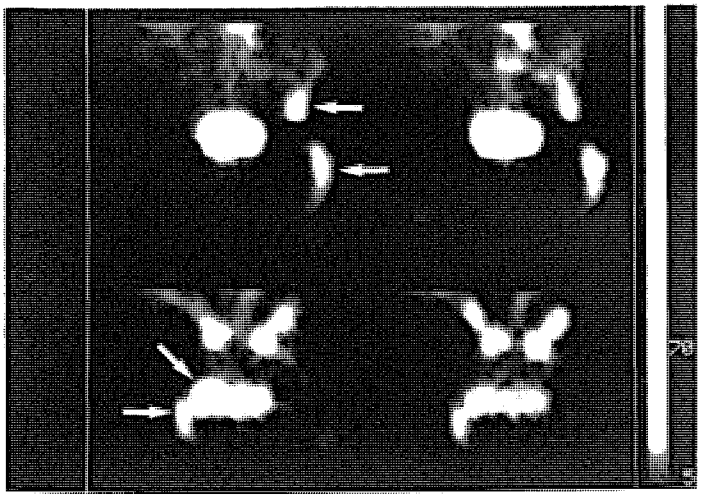

FIGURE 2b:
Coronal SPECT slices 6 h post injection: same patient as figure 2a.

Table 5
Total and antiisotypic HAMA factors (IgG, IgM) after repeated application of 0.3 mg MAb BW 250/183 in a patient with positive response

	total		iso	
weeks	IgM	IgG	IgM	IgG
0 (IS)	4.65	1.52	4.65	1.42
3	7.24	2.12	6.75	2.28
6 (IS)	6.64	3.44	5.04	2.06
9	6.06	8.12	4.99	3.08
12 (IS)	6.34	9.38	3.59	3.30
15	6.07	13.83	4.70	3.70

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