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Development of human anti-murine antibody (HAMA) response in patients

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Summary Human anti-mouse antibody (HAMA) response was determined in the serum of 67 patients who received subcutaneously administered radiolabelled murine monoclonal antibodies (MoAb) (50 μg–3 mg) for immunolymphoscintigraphy and of 10 patients with advanced colorectal cancer who received murine MoAb-*N*-acetyl melphalan (MoAb-*N*-AcMEL) conjugates (amount of MoAb ranged from 120 mg/m^2 body surface area to 1000 mg/m^2 body surface area) as therapy. A pre-existing low level of apparent human anti-mouse antibody reactivity could be detected in the serum of normal subjects and patients prior to administration of murine MoAb. Subcutaneous administration of low doses of murine MoAb, as used in immunolymphoscintigraphy, was associated with a low incidence (4/67 or 6%) of elevated HAMA response; the use of F(ab′)$_2$ fragments was associated with the development of elevated HAMA response in one of three patients. By contrast, therapy with hepatic artery infusion of murine MoAb-*N*-AcMEL conjugates in three repetitive daily doses (each infusion lasting 2 h) elicited elevated HAMA responses in 10/10 (100%) patients, usually 1–3 weeks after the start of therapy. The HAMA response of patients in the therapy group was higher than those in the immunolymphoscintigraphy study and the use of steroids did not prevent the development of the HAMA response. Further administration of MoAb-*N*-AcMEL conjugates to a patient, who had already developed HAMA, led to 'serum sickness'-type symptoms and a transient reduction in the HAMA titres. The elevated HAMA response was polyclonal, containing increased levels of both immunoglobulin M and G (IgM and IgG) and was directed against mouse-specific determinants, the isotype (presumed to be the Fc portion), the F(ab′)$_2$ and the 'idiotype' of mouse immunoglobulins.

INTRODUCTION

Murine monoclonal antibodies (MoAb) with specificity for tumour-associated antigens are increasingly being used as carrier molecules for radio-imaging agents such as ^{131}I, ^{111}In, ^{123}I (1–4) and for therapeutic agents such as cytotoxic drugs (5–7). One problem with the use of murine MoAb in humans has been the development of the human anti-murine antibody (HAMA) response (8–11) which restricts repetitive dosing. However, reports differ on the incidence and nature of the development of HAMA response; the differences were probably related to the different clinical programmes (amount of foreign protein administered, the route and number of treatments and the time interval between treatments), the immune status of the patients and the methodological differences in assay techniques for HAMA. In addition, the nature of HAMA response in cancer patients may be different from that elicited in transplant patients by mouse MoAb OKT3 and this is not surprising as OKT3 has both stimulatory and suppressive effects on T cells (12).

Radio-iodinated murine MoAb RCC-1 (reactive with breast cancer) had been administered subcutaneously (s.c.) for immunolymphoscintigraphy, and when used together with cold iodine labelled 'blocking' Ly2·1 antibody (non-reactive with breast cancer), successful localization of axillary lymph node metastases from breast cancer occurred in about 90% of cases (3). Various MoAb have also been conjugated to the *N*-acetyl derivative of melphalan (*N*-AcMEL) and shown to have *in vitro* and *in vivo* specificity and cytotoxicity and specifically inhibit the growth of

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Abbreviations used in this paper: ^{131}I, iodine-131; HAMA, human anti-murine antibody; i.v., intravenous; w/w, weight:weight; Ig, immunoglobulin; MoAb, monoclonal antibody/ies; PBS, phosphate buffered saline; s.c., subcutaneous(ly).

human colon carcinomas xenografted in athymic mice (13,14). The MoAb-*N*-AcMEL conjugates have also been administered by means of hepatic artery infusion to patients with advanced colorectal cancer and have achieved biological responses in some patients (7).

The aim of the study was to determine the nature of the HAMA response in patients after regional administration of murine MoAb for immunolymphoscintigraphy (s.c. injection) or therapy (hepatic artery infusion).

MATERIALS AND METHODS

Murine monoclonal antibodies

For imaging studies, murine MoAb 3E1·2 immunoglobulin (IgM), RCC-1 (formerly called 17·1; IgG 2a) and Ly2·1 (IgG2a) were used. Antibody 3E1·2 was raised against fresh human breast carcinoma (15). RCC-1 was raised by immunizing inbred Biozzi mice with the MCF-7 breast cancer cell line (16). The Ly2·1 antibody is reactive with the murine Ly2·1 specificity but not with human breast cancer (17). The Ly2·1 antibody was used in the study as a 'blocker' to reduce background non-specific uptake of ^{131}I-RCC-1 (3). Murine MoAb used for therapy included 30·6 (IgG2b), which is reactive against a large number of colon carcinoma cell lines (18), I-1 and JGT (IgG1), which are both anti-CEA (carcinoembryonic antigen) (19). Irrelevant murine MoAb used to evaluate the HAMA response included BC1 (IgG3), BC2 (IgG1), and BC3 (IgM) which were directed against mucin-like glycoproteins of breast (20); and polyclonal antibodies of monkey and sheep origin (produced in our laboratory). MoAb 3E1·2 was purified from ascitic fluid (21) and the antibodies RCC–1 and Ly-2·1 were purified on Protein A-Sepharose (Pharmacia Inc., Piscataway, New Jersey, USA as described previously (22); antibodies 30·6, I-1 and JGT by Protein A-Suparose (Pharmacia Inc., Piscataway, NJ). Purity was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The F(ab′)$_2$ fragments were obtained from purified MoAb RCC-1 by pepsin digestion (23). Antibodies were aliquoted and stored at −70°C until used.

Iodination of monoclonal antibodies

Purified antibodies (3E1·2, RCC-1 or RCC-1 F(ab′)$_2$) were radiolabelled with ^{131}I (Amersham Int., UK) using the iodobead (24) or enzymobead reagent (Bio-Rad, Richmond, California, USA) (25). The purified Ly2·1 antibody was labelled with non-radioactive sodium iodide or ^{131}I using the chloramine T method (26). Preparation of the iodinated antibodies has been previously described (3,21).

Preparation of drug-antibody conjugates

The *N*-acetyl derivative of melphalan (*N*-AcMEL) was prepared and conjugated to the antibodies (30·6, I-1 and JGT) as described previously (14). The antibody activity and cytotoxicity of the immunoconjugates were ensured by resetting assay (27) and inhibition of DNA synthesis using [^{3}H]-thymidine, respectively (14).

Patient studies

For diagnostic studies (immunolymphoscintigraphy) to localize axillary lymph node metastases, 67 patients with various breast conditions (benign and malignant) (Table 1) received a variety of iodinated preparations s.c. They included ^{131}I-labelled 3E1·2 (50–200 μg), ^{131}I-labelled RCC-1 (50–400 μg), ^{131}I-labelled Ly-2·1 (0·4–2 mg), ^{131}I-labelled RCC-1 (0·4–1 mg) together with 'blocker' antibody Ly2·1 (2 mg) iodinated with non-radioactive sodium iodide and ^{131}I-labelled RCC-1 F(ab′)$_2$. To prevent thyroid uptake of free radioiodine, patients received potassium iodide (5 mL of 16·54% w/v) and sodium perchlorate (400 mg) orally, 1 h before the s.c. injection; the potassium iodide was continued for 5 days after the injection.

For therapy, nine patients with extensive colorectal hepatic metastases (2/9 also had pulmonary metastases) and one patient who had a curative resection of the Duke's C colon cancer received between 120 mg/m^2 and 1000 mg/m^2 body surface area of MoAb (274–1696 mg) (I-1 and/or JGT and/or 30·6) conjugated with between 5 mg/m^2 body surface area to 20 mg/m^2 body surface area of *N*-AcMEL (Table 2). The MoAb selected for conjugation with *N*-AcMEL were, where possible, individually chosen for each patient, based on the binding of the particular antibody (I-1, JGT, 30·6) to sections of the primary colon cancer tissue as assessed by immunoperoxidase staining. In general, MoAb was selected only if it stained >50% of the carcinoma cells on the sections. None of the patients received any other form of treatment for 4 weeks before and 8 weeks after treatment with the immunoconjugates.

The immunoconjugate was administered via hepatic artery infusion over 2 h per day for 2 days (7). All patients received the immunoconjugates in three equal doses (t = 0 h, t = 24 h, t = 48 h). Patients had prophylactic intravenous (i.v.) dexamethasone 8 mg just before each infusion of the immunoconjugate and oral prednisolone 10 mg daily for 7 days after completion of infusion.

Both studies were approved by the Medical Research Board of the Royal Melbourne Hospital and written informed consent was obtained from every patient. Blood samples were obtained from patients before, during and after the injection of MoAb conjugates to detect human anti-murine immunoglobulin (HAMA) response. Normal serum samples (n=20) were also obtained from apparently healthy blood donors. All samples were aliquoted and stored at −70°C.

Human anti-murine antibody (HAMA) response

Human antibodies against the murine MoAb were measured by an enzyme-linked immunosorbent assay (ELISA), modified from that described previously (9). Ninety-six well flexible polyvinylchloride (PVC) plates (Costar, Cambridge, Massachusetts, USA) were

coated with 100 μL/well of various purified murine MoAb (5μg/mL) in a 0·1 M carbonate buffer, pH 9·6 and non-specific binding blocked with 1% bovine serum albumin/PBS (phosphate buffered saline) pH 7·6. Serial dilutions of patients' sera and pooled normal human serum (50 μL/well) in PBS/0·05% Tween 20 were added to the antibody coated wells and incubated for 16 h at 4°C. Plates were then washed with PBS/0·05% Tween 20 and then reacted with 50 μL/well of a 1:600 dilution of sheep anti-human immunoglobulin conjugated to horseradish peroxidase (Amersham International, UK) for 3 h at 37°C. The colour reaction was developed using 50 μL of 0·03% ABTS [2,2′-azino-di- (3-ethylbenthiazoline) sulfonate] (Amersham International, UK) and 0·02% hydrogen peroxide (BDH Chemicals, Poole, UK) and read with an ELISA plate reader (Titertek Multiscan MC) at a wavelength of 405 nm. In some cases, the IgM and IgG components of the HAMA response were separately measured by using phosphatase labelled affinity purified goat anti-human IgM or IgG (Kirkegaard and Parry, Maryland) respectively and the colour reaction developed with alkaline phosphatase substrate. Results were expressed as the absorbance value of patient serum compared with control serum (pooled normal human serum from 20 apparently healthy blood donors) and a positive test was defined as one in which the absorbance was equal to or greater than twice the absorbance of pooled normal human serum. The HAMA titre was determined by obtaining the inverse of the highest serum dilution which gave a positive test result, and was arbitrarily graded as weak (titre 100–<1600), moderate (titre 1600–6400) or strong (titre > 6400). The background was too high at serum dilution less than 1/100; thus, within the sensitivity of the assay, HAMA titre of less than 100 cannot be determined accurately.

RESULTS

Imaging studies

A low level of apparent anti-murine immunoglobulin activity can be measured in the serum of normal individuals (NHS) (Fig. 1, NHS) and also in the pre-immune serum of patients. These levels were detected both in patients who later produced an elevated HAMA response and in those who did not produce such a response. A HAMA response was considered positive when the absorbance of the serum was equal to or greater than twice the absorbance of pooled normal sera (Fig. 1). Table 1 summarizes the measurements of antibody to murine antibodies in 67 patients with various breast conditions (benign and malignant) prior to subcutaneous injection of murine antibodies for imaging studies and 2 weeks–2 months after injection. Four of 67 patients (6%) showed a positive HAMA response (Table 1), which developed within 2 months of exposure. None of the patients had a strong response (titre > 6400) and in the limited number of patients studied, the intensity of the response did not relate to the amount (50 μg–3 mg) of murine antibody injected. In addition, none of the patients developed any clinical side effects from the injection that could be related to the development of a positive HAMA response. Thus doses of up to 3 mg of murine MoAb given s.c. did not give rise to an elevated HAMA response in most patients.

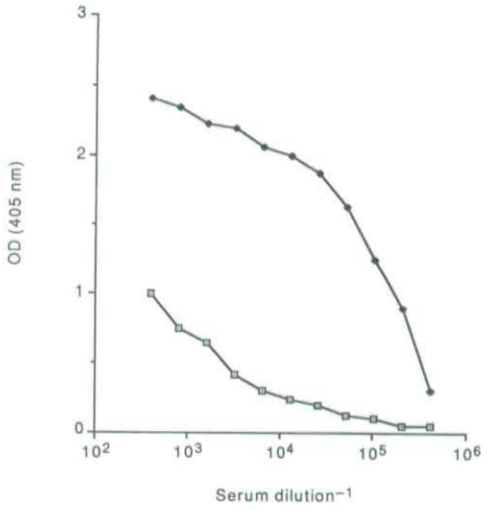

Fig. 1. Level of pre-existing anti-murine antibody in a representative pooled normal human serum (⊡) compared with the elevated HAMA response in a patient (●) (Table 2; Patient 7) who had received therapeutic administration of murine MoAb-*N*-AcMEL conjugates 4 weeks previously.

Therapy studies

Table 2 summarizes the HAMA response in ten patients with advanced colorectal cancer prior to and after therapy with MoAb-*N*-acetyl melphalan conjugates (amount of antibody: 120 mg/m^2–1000 mg/m^2) via hepatic artery infusion. All the patients had three doses of the injection, 24 h apart and received prophylactic steroid therapy 1 h before and for 7 days after therapy. All the ten patients (100%) developed elevated HAMA responses; the intensity of the response did not correlate with the amount of murine antibodies received (Table 2) or with the pre-immune level of anti-murine immunoglobulin activity. The elevated HAMA response

Table 1. Summary of HAMA response in patients receiving murine MoAb for imaging studies.

MoAb administered (amount)	Time from administration of Ab	No. of patients studied	Number of patients with HAMA response (titre)			
			Nil (<100)	Weak (100–<1000)	Moderate (1000–6400)	Strong (>6400)
3E1·2 (50–200 μg)	0	12	12	0	0	0
	2 weeks–2 months		11	1	0	0
RCC-1 (50–400 μg)	0	17	17	0	0	0
	2 weeks–2 months		16	0	1	0
RCC-1 (400 μg–1 mg)	0	32	32	0	0	0
+Ly-2·1 (2 mg)	2 weeks–2 months		31	0	1	0
Ly-2·1 (400 μg–2 mg)	0	3	3	0	0	0
	2 weeks–2 months		3	0	0	0
RCC-1 $F(ab')_2$ (400 μg)	0	3	3	0	0	0
	2 weeks–2 months		2	0	1	0

Table 2. Summary of therapy studies.

Patient	MoAb administered	Amount of Ab[a]	Time from administration of antibody (weeks)	HAMA response (titre)
1	I-1	120 mg/m² (R×1)	0	<100
			4	$1·5\times10^5$
			8	$1·0\times10^5$
	I-1, JGT	160 mg/m² (R×2)	0	$1·0\times10^5$
			4	$1·9\times10^5$
			8	$1·5\times10^5$
2	30·6, I-1, JGT	980 mg/m²	0	<100
			4	$5·0\times10^4$
			8	$2·5\times10^4$
3	I-1	250 mg/m²	0	<100
			4	$2·0\times10^4$
			8	$1·5\times10^4$
4	30·6, I-1, JGT	340 mg/m²	0	<100
			4	<100
			8	$3·2\times10^3$
5	I-1	380 mg/m²	0	<100
			4	$1·0\times10^5$
			8	$5·0\times10^4$
6	I-1, JGT	500 mg/m²	0	<100
			4	$5·0\times10^4$
			8	$5·0\times10^4$
7	30·6	440 mg/m²	0	<100
			4	$4·0\times10^5$
			8	$2·0\times10^5$
8	30·6, I-1, JGT	1000 mg/m²	0	<100
			3	$1·0\times10^5$
9	I-1, JGT	820 mg/m²	0	<100
			4	$3·2\times10^3$
			8	$3·2\times10^3$
10	I-1, JGT	1000 mg/m²	0	<100
			4	$5·0\times10^4$

[a]When multiple antibodies were used for drug conjugation, the final preparation of the immunoconjugates had, where possible, equal proportions of each MoAb. Doses were expressed in amount of MoAb/surface area of the patient. R×1, first course of treatment; R×2, second course of treatment, given to Patient 1.

developed in 1/10 patients as early as Day 5 after the first administration of infusion, but most patients (7/10) developed elevated HAMA response (as defined earlier) between 1 and 3 weeks after the exposure to the foreign protein (Fig. 2). The peak response occurred 14–28 days following exposure in 7/8 cases; in two other patients, serum beyond this period was not available. In 2/3 patients who were followed for up to 9 months, the elevation of HAMA response persisted but there was a gradual fall in the titres. Five of the patients (Table 2; Patient 1 R×2, patients 7, 8, 9 and 10) developed fever and this appeared to correlate with the amount of the immunoconjugates received and was not related to the intensity of the HAMA response. One patient (Patient 1) received 120 mg/m^2 of MoAb and developed an elevated HAMA response (Fig. 3). Two months later a further dose (160 mg/m^2 MoAb) was given and symptoms suggestive of a Type III hypersensitivity 'serum sickness' reaction developed but was self limiting. During the second course of treatment (Fig. 3, R×2), there was an initial reduction of HAMA titre which might indicate immune complex formation, followed by a boost of the HAMA response, as from Day 10 onwards.

Specificity of HAMA response

To determine whether the HAMA response was directed against common determinants on the constant domain of mouse immunoglobulins or whether it was specific for each MoAb, the binding of patients' sera to microtitre wells containing relevant MoAb (i.e., the same as that injected into patients), or other murine MoAb ('irrelevant') of the same or different isotypes as that of the relevant antibody were assayed as before. The results of a representative patient (Table 2; Patient 1) are shown. As shown in Fig. 4(a), sera from Patient 1 in the therapy studies, who had a positive HAMA response, contained antibodies that bound well to both the relevant (injected: I-1) and the irrelevant murine MoAb (3E1·2, BC2), irrespective of antibody subclass, Similar results were obtained when the serum samples of other patients who developed a positive HAMA response were tested.

This indicates that there is a major reaction to common determinants of all mouse immunoglobulins; such a reaction could be with the kappa light chain, but this was not specifically measured.

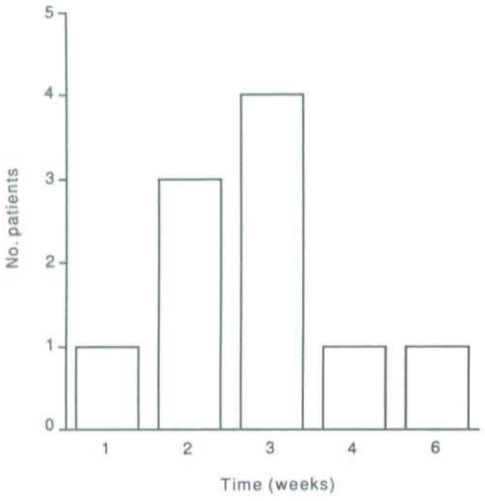

Fig. 2. Time in weeks after initiation of MoAb-*N*-AcMEL treatment when elevated HAMA responses were first detected. Week 1 refers to days 1–7 after the start of treatment; Week 2 refers to days 8–14, and so on.

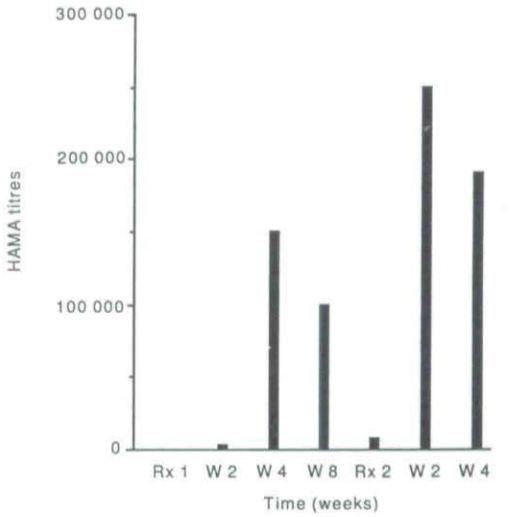

Fig. 3. Development of elevated HAMA response in Patient 1 (Table 2) after the first treatment (R×1) of MoAb-*N*-AcMEL with time. Changes in levels of free circulating HAMA during (R×2) and after second administration of MoAb-*N*-AcMEL were also illustrated. The time was expressed in weeks (W) from the time of respective treatment.

The binding of sera of patients, before and after immunization, to polyclonal antibodies of monkey and sheep origin coated on the plate was also assessed in a direct binding assay. Data from a representative patient (Table 2, Patient 1) are presented in Fig. 4(a). There was detectable antibody response towards immunoglobulin preparations of monkey and sheep in the 'pre-immune' serum but the elevated antibody response after therapy was directed predominantly against determinants unique to murine immunoglobulin preparations. Similar results were obtained when the sera of other patients who had a positive HAMA response were tested.

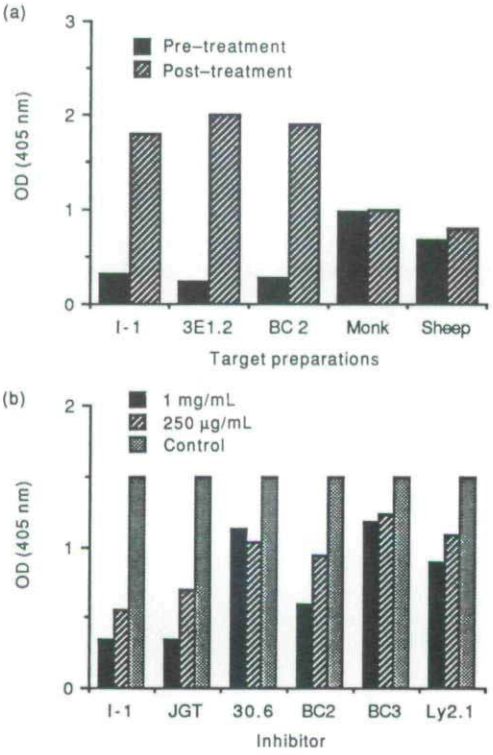

Fig. 4. (a) Reaction of HAMA in the serum of a representative patient (Table 2: Patient 1) with various murine MoAb, relevant (I-1:IgG1) and irrelevant (3E1·2: IgM, BC2: IgG1) and to polyclonal antibodies from monkey (Monk) and sheep. The various antibodies were coated on the plate and solid phase binding assays performed similar to HAMA assay; a 1:2000 dilution of serum was used. The binding of pre-existing HAMA in the same patient to the various antibodies was also shown.

(b) Inhibition of HAMA activity by different murine MoAb preparations. Serum of Patient 1 was incubated with relevant MoAb (I-1, JGT; both IgG1) or irrelevant murine MoAb of different isotypes (30·6: IgG2b, BC2: IgG1, BC3: IgM, Ly 2·1: IgG2a) in different concentrations (250 µg/mL or 1 µg/mL) and tested in HAMA assays by measuring the binding of the reaction mixture to MoAb I-1 coated plate. Only the results of a representative patient (Patient 1) are shown, for clarity. Results were compared to the binding of the same respective serum sample in HAMA assay in the absence of a blocking antibody (control).

A competitive inhibition ELISA, performed on immune serum from Patient 1 showed that MoAb I-1 and JGT (relevant MoAb) competed for I-1 binding sites more efficiently than the irrelevant murine MoAb of the same (IgG1: BC2) or different (IgG2a: Ly-2·1; IgG2b:30·6; IgM: BC3) isotypes (Fig. 4b). However, all the murine MoAb inhibited the binding of human anti-murine antibody to murine antibody (coated on the plate) to some extent. Similar results were obtained using sera from other patients with positive HAMA response in both the therapy and the imaging studies (data not shown). In these inhibition studies, which are analogous to an absorption test, it was clear that the best inhibition by irrelevant antibody in the case of Patient 1 (Fig. 4b) was by antibody BC2 that was of the IgG1 isotype (same isotype as the administered antibodies) and this inhibited better than irrelevant IgG2a, IgG2b and IgM antibodies. It was concluded that part of the HAMA response was anti-isotype specific, presumably to the Fc region of the IgG1 immunoglobulin. The fact that inhibition was best using the relevant (administered) antibody I-1 or JGT, indicated that some of the immune response was specific to the administered antibodies. This latter response is called, in various studies, 'anti-idiotypic' (10,28) but we have not conducted the appropriate studies to determine whether this component of the HAMA response is to the antigen binding site or to other sites on the murine antibody molecules. Thus the HAMA response in the patients studied had three components: (i) anti-mouse immunoglobulin (mouse-specific determinants: heavy and/or light chains); (ii) anti-isotype; and (iii) antibody response specific to the administered mouse immunoglobulin (loosely termed 'anti-idiotype' in this study).

In addition, the IgM and IgG components of the anti-murine immunoglobulin activity could be separately measured in the 'pre-immune' human serum (Fig. 5) and each component was increased with the development of elevated HAMA response. It is clear that the HAMA response was essentially polyclonal and contained increased levels of IgM as well as IgG.

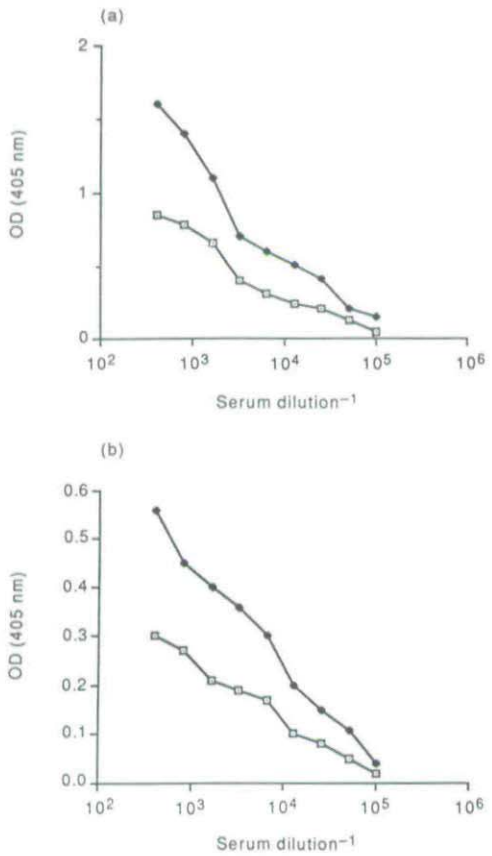

Fig. 5. Levels of (a) IgG and (b) IgM HAMA detected in the serum of a representative patient (Table 2: Patient 6) before (□) and after (●) therapeutic injection of MoAb-*N*-AcMEL conjugates.

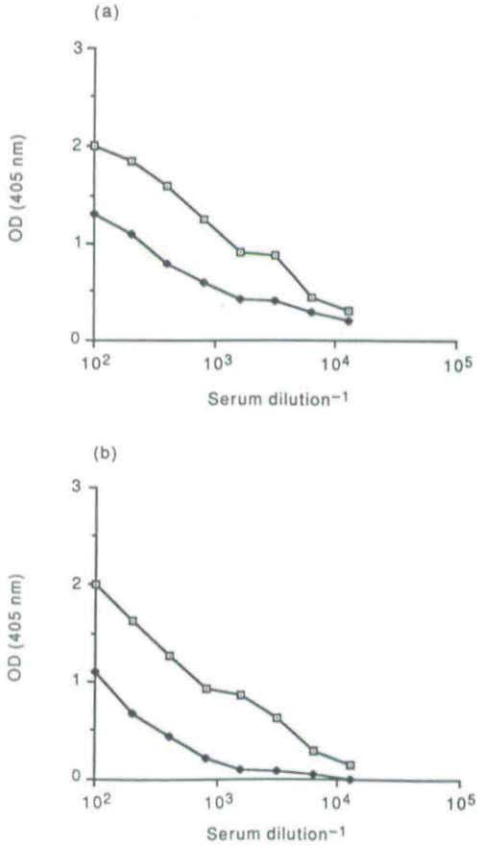

Fig. 6. Elevated HAMA responses made by a representative patient (from imaging studies) to whole IgG (■) and F(ab′)$_2$ (●) of (a) relevant (RCC-1) and (b) irrelevant (Ly-2·1) murine MoAb; both were of IgG2a subclass. Relevant antibody refers to the administered antibody.

HAMA response to Fc and F(ab′)$_2$ of mouse immunoglobulin

To determine the relative contribution in patients' sera of antibodies to Fc and F(ab′)$_2$ portions of the injected murine immunoglobulin, ELISA plates were set up with whole IgG MoAb, or with equimolar amounts of the F(ab′)$_2$ fragments of the same immunoglobulin; and the binding of patients' sera to these different preparations was assayed using serum samples with an elevated HAMA response. In a representative patient (Fig.6), the serum displayed elevated responses to both the whole murine immunoglobulins and the F(ab′)$_2$ fragments. The reactions to the whole IgG of both relevant (Fig. 6a) and irrelevant (Fig. 6b) antibodies were greater than to F(ab′)$_2$ fragments, although this could reflect differences in the amount of antigen bound. The pre-immune sera also displayed a similar pattern of reaction, although the binding was less. Thus the HAMA response was directed against both the Fc and F(ab′)$_2$ of mouse immunoglobulin.

DISCUSSION

The HAMA responses in patients who received murine MoAb for diagnostic studies or for therapy were examined in this study. Immunolymphoscintigraphy, using subcutaneously administered antibodies in amounts ranging from 50 μg–3 mg was clinically safe (3,21) and was associated with a low incidence (4/67) of

HAMA, as measured by the ELISA system used in the study. In addition, the degree of response tends to be mild to moderate (HAMA titres 100–6400), as defined in the study. This is in contrast to the higher incidence of HAMA observed with some studies of immunoscintigraphy which employed larger doses (0·16–20 mg) of murine MoAb injected intravenously (29) or indeed with the larger amounts of antibody given intra-arterially in this study. The difference in immune response may be because the dose used was small (50 μg–3 mg) and was injected subcutaneously rather than intravenously. A similar lack of elevated HAMA response had been reported by others who used small doses (250 μg) of murine antibodies injected intravenously (8). However, there are other possible explanations, such as ^{131}I-MoAb being taken up in B cells which may be inactivated by the 'hot' antigen, although such an inactivation did not occur after intravenous use of radiolabelled antibodies (29). Another major difference for consideration is that the melphalan–antibody conjugate could be more immunogenic than either MoAb or ^{131}I-MoAb used in the imaging procedure; we have no information on whether this is the case. However, the low incidence of HAMA response in imaging is of practical importance as it indicates that such diagnostic procedures can be repeated in most patients and do not preclude future therapy with murine MoAb.

By contrast, hepatic artery infusion of murine MoAb-*N*-acetyl melphalan conjugates in three repetitive doses over 48 h elicited prompt and dramatic immune response to the murine immunoglobulins in all ten patients. An elevated HAMA response was noted in a patient as early as the fifth day following exposure to the foreign protein; however, the peak values of HAMA response usually occurred 14–28 days following exposure. The degree of HAMA response did not correlate with the amount of murine antibodies received within the dose range of 120–1000 mg/m^2 (Table 2), and there was no correlation between the degree of immune response in the patients and the clinical response (7). The high incidence of elevated HAMA response in this group of patients could be related to the larger doses used, its repeated (three doses) daily administration and the route of adminstration by hepatic artery infusion. This is in accord with the high incidence of HAMA (17/18 patients) that was detected after repeated daily i.v. infusions of an IgG1 antibody in patients with pancreatic cancer (28). It is of interest that the frequency of HAMA had been noted to be lower (50%) with single infusions of murine antibody 17-1A (IgG2a) (30). Although others had reported a reduction of this anti-murine response when patients received high initial doses of MoAb 17-1A (700 mg) by i.v. infusions, and had attributed that to induction of immune tolerance (31), this was not the experience of this study. The repeated daily administrations of the immunoconjugates via hepatic artery infusion before the development of detectable elevated HAMA response were well tolerated; by contrast, injection of the immunoconjugates in the presence of an elevated HAMA response was associated with an initial reduction in the HAMA titres which probably corresponded to immune complex formation and with clinical symptoms suggestive of a Type III hypersensitivity 'serum sickness' reaction, followed by a boost in the HAMA response (Fig. 3). The presence of an elevated HAMA response therefore precludes any further exposure to murine immunoglobulins. Where increased anti-murine antibody levels were detected after exposure to murine antibodies either for imaging or for therapy, the HAMA response was polyclonal, containing increased levels of both IgM and IgG and was directed against mouse-specific determinants, the isotype (presumed to be the Fc portion), the F(ab′)$_2$, as well as the 'idiotype' (i.e. anti-individual injected antibody) of mouse immunoglobulin. The contradictory reports on the nature of the HAMA response, especially with regard to the 'anti-idiotypic' component could be related to the different assay systems used to evaluate the response (8,10,32,33).

The low level of human anti-murine antibody response detected in the serum of normal subjects and in patients prior to administration of murine MoAb, is in accord with other studies (8–10) and there is evidence that this at least in part reflects rheumatoid factor activity in normal human sera and not a pre-existing specific antibody for murine immunoglobulin (34). It appeared that a significant component of the elevated HAMA response in patients given murine MoAb represented secondary response to antigenic determinants common to mouse immunoglobulins and some unknown immunogen, to which low levels of sensitization had already occurred. However, the predominant component of the elevated HAMA response was directed against mouse-specific determinants (Fig. 4a) and it is generally conceded (35) that the antibody response developed by patients was specifically related to the species from which the administered antibody was derived.

The findings of this study have several clinical

implications. First, as the human anti-murine antibody response was polyclonal and was directed against the antigenic determinants on the constant, Fc, as well as the $F(ab')_2$ portion of the mouse immunoglobulin molecule, the use of $F(ab')_2$ fragments would not confer a real advantage over intact IgG, as illustrated by the elevated HAMA response in 1/3 patients given $F(ab')_2$ in imaging studies (Table 1). Second, the administration of murine antibodies subcutaneously in doses up to 3 mg was associated with a low incidence (4/67 or 6%) or elevated HAMA response and thus would not, in most cases, preclude subsequent exposure to murine antibodies. Third, repeated (three doses) daily administrations of murine antibodies over 48 h in doses up to 1000 mg/m^2 by hepatic artery infusion was not associated with untoward complication, provided there was no prior immunization to murine immunoglobulins (7). Finally, the use of steroids in the peri-therapy period did not prevent the development of 'positive' HAMA response.

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