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Human anti-mouse antibody response to the injection of murine monoclonal antibodies against IL-6

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SUMMARY

We analysed human anti-mouse antibodies (HAMA) in 12 patients (six with multiple myeloma (MM) and six with metastatic renal cell carcinoma (MRCC)) who were treated with B-E8, an IgG1 MoAb against IL-6. Efficiency of the treatment was evidenced by the drop in the serum levels of C-reactive protein (CRP), the *in vivo* production of which is under the control of IL-6. Three patients with MM and the six patients with MRCC became immunized to the injected MoAb. HAMA appeared between days 7 and 15 after the beginning of the treatment. The nine patients made IgG antibodies; four also made IgM. All immunized patients made anti-idiotype antibodies specific to B-E8. Two of them also developed HAMA directed to murine IgG1 isotype; in these two patients B-E8 MoAb cleared rapidly from the circulation with loss of treatment efficiency. In the patients who developed only anti-idiotype antibodies, serum levels of B-E8 remained unchanged and CRP production remained inhibited, indicating that treatment remained efficient in the presence of HAMA. Circulating B-E8 MoAbs were still able to bind to IL-6 and to inhibit IL-6-dependent proliferation despite the presence of anti-idiotypic HAMA. Therefore, in contrast to HAMA produced against MoAb directed against cellular targets, HAMA against anti-IL-6 MoAb idiotopes led neither to clearance nor to functional inactivation of the injected MoAb. This was further shown by resuming the B-E8 treatment with success in a patient who still had anti-idiotypic HAMA.

Keywords cancer immunization immunotherapy IL-6 monoclonal antibodies

INTRODUCTION

MoAb-based immunotherapy ideally targets pathological effectors, and is particularly well adapted when cells or protein factors need to be inactivated. A major limitation of the use of murine MoAb is the human anti-mouse immunoglobulin antibody (HAMA) response, which occurs frequently [1–4]. HAMA rarely lead to hypersensitivity reactions, but frequently cause inactivation of the injected MoAb. The phenomenon has been widely studied in therapies against cellular targets for eradication either of immunocompetent lymphocytes to achieve immunosuppression [4], or tumour cells [3]. For example, transplanted patients treated with CD3 MoAb rapidly produced IgM and IgG antibodies to either isotypic or idiotypic determinants of the CD3 MoAb; only anti-idiotypic IgG antibodies led to inactivation of CD3 MoAb [5,6].

Targeting of cytokines by MoAb is becoming an interesting method of immunotherapy. Since the HAMA response against MoAb targeting soluble antigens may be different from the former situation, we studied immunization against anti-IL-6 murine MoAb. IL-6 is a multi-functional lymphokine which has recently been shown to exert growth factor activity for various tumour cells such as plasma cells in multiple myeloma (MM) [7,8] or tumour renal cells in metastatic renal cell carcinoma (MRCC) [9]. MoAbs against IL-6 were used for treating patients with MM [10] and were shown to interrupt the growth of the tumour cells. Such MoAbs were also used for treating patients with MRCC. We describe here the kinetics of the immunization against anti-IL-6 MoAb, the type of HAMA produced and the possible deleterious effects of these antibodies.

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PATIENTS AND METHODS

Description of the clinical study

Pretreatment characteristics of the patients are summarized in Table 1. Six patients with advanced and progressive MM refractory to standard chemotherapy were treated with anti-IL-6 MoAb. Their life expectancy was judged to be less than

Table 1. Pretreatment characteristics of the 12 patients

Patients	Pathology	Sex/age (years)	Pretreatment CRP (mg/ml)	Previous treatment
1	MM	F56	90	C
2	MM	M65	123	C
3	MM	F45	103	C, S
4	MM	M61	70	C
5	MM	M59	60	C
6	MM	F55	21	C, I, R
7	MRCC	M63	75	I, S
8	MRCC	M72	140	I, S
9	MRCC	M70	216	I
10	MRCC	M42	120	I, S
11	MRCC	M78	8	S
12	MRCC	M70	143	S

MM, Multiple myeloma; MRCC, metastatic renal cell carcinoma; F, female; M, male; C, chemotherapy; I, immunotherapy (IFN-α and/or IL-2); R, radiotherapy; S, surgery.

1 month. Briefly, in three patients (patients 2, 4, and 6) the treatment resulted in an inhibition of myeloma cell proliferation, whereas no anti-tumour response was observed in patients 1 and 3 [10,11]. In patient 5 a six-fold decrease of circulating plasma cells was seen within the first 8 days of the treatment, followed by a dramatic increase after day 9. Six patients with MRCC (mean age 65·8 years) were treated with anti-IL-6 MoAb [12]. Four of them had already been treated with interferon-alpha (IFN-α) and/or IL-2; two had only been subjected to surgery. No obvious anti-tumour response was noticed in the patients. However, a minor reduction of adenopathies (patient 11) and of the tumoral thrombus present in the vena cava of patient 9 were seen. In all patients inhibition of toxicities related to IL-6 overproduction were observed. A striking, rapid decrease of fever and pain led some patients to abandon the intake of major analgaesic drugs. No major toxicity effects were seen. Analysis of the effects of the IL-6 therapy in the case histories of the patients will be submitted. These clinical trials were carried out with the approval of the local ethical committee of the Centre Hospitalier Regional de Montpellier. Plasma samples were obtained by centrifugation of heparinized venous blood collected in the morning before MoAb injections, and stored frozen at −20°C.

Anti-IL-6 MoAb

The anti-IL-6 MoAbs B-E4 (IgG2b) and B-E8 (IgG1) were prepared by J.W. by hybridizing spleen cells from mice immunized with recombinant IL-6 (rIL-6) with X63/Ag86553 myeloma cells [13]. These antibodies were directed against two different epitopes of IL-6. They were purified from ascites and checked for absence of virus, bacteria and pyrogenicity, these being the analyses legally required before being used as previously described [10]. CD1, BL6 and CD37, BL14 (IgG1), and CD8, BL15 (IgG2b) had been prepared by J.B. and were used as control MoAbs. For the study, all MoAbs were purified by affinity chromatography on Protein A-Sepharose, eluted at acid pH and dialysed against PBS. Murine albumin was purchased from Sigma (St Louis, MO).

ELISA techniques

Levels of circulating anti-IL-6 MoAb were determined by an assay detecting mouse immunoglobulin. Polystyrene plates (Immuno I; Nunc, Kamstrup, Denmark) were coated overnight at 4°C with purified goat IgG against mouse immunoglobulin (Jackson Immunoresearch, West Grove, PA; 10 μg/ml in PBS). Plates were then saturated with 1% bovine milk proteins (BMP) in PBS for 1 h at room temperature. After five washings in PBS containing 0·05% Tween 20 (Sigma), 100 μl of the patients' plasma diluted in PBS-BMP-Tween (1% BMP in PBS containing 0·05% Tween 20) were added for 1 h at room temperature. Plates were washed five times with PBS-Tween before a solution containing peroxidase-conjugated goat anti-mouse immunoglobulin light chains (Tago Inc, Burlingame, CA) was added for 1 h, and then they were washed again another five times. The reactivity was determined by the intensity of the enzymatic reaction to the substrate *o*-phenylenediamine (OPD) measured by absorbance at 492 nm (Titertek Multiskan MC; Flow, Irvine, UK) after addition of 50 μl 2 N H_2SO_4. Mouse immunoglobulin concentrations were calculated by using a reference curve obtained from serial dilutions of B-E4 or B-E8 MoAbs.

Presence of human antibodies to anti-IL-6 MoAb was checked by another ELISA. Plates (Immuno I; Nunc) were coated overnight at 4°C with either 10 μg/ml in PBS of either B-E4 or B-E8, or a control MoAb of the same isotype as B-E4 (CD8, BL15 IgG2b) or B-E8 (CD37, BL14 and CD1, BL6 as IgG1). We checked that the plates were coated with the same amounts of each MoAb by using a peroxidase-conjugated goat anti-mouse immunoglobulin (Tago). Plates were saturated with PBS-BMP as above and the patients' plasma samples (diluted 1 : 100 in PBS-BMP) were added for 1 h at room temperature. The human immunoglobulins were detected by incubating plates with peroxidase-conjugated goat anti-human IgG or IgM serum (Jackson Immunoresearch), then OPD. Amounts of detected HAMA were expressed in absorbance units. In the absence of a standard source of human anti-B-E8 antibodies, no direct method allowed the measure of their concentration. To ensure that IgG concentration was linearly proportional to absorbance, we coated varying amounts of purified human IgG on immunoplates and then added the peroxidase-conjugated goat anti-human IgG serum (Jackson Immunoresearch) as above; a linear relationship was seen when OD values were $< 1{\cdot}6$.

Inhibition studies of HAMA. In the ELISA used for detection of HAMA, we added patients' plasma pre-incubated with various amounts of B-E8 or of MoAbs of various isotypes in order to produce competitive inhibition with the MoAb used for coating the immunoplates.

Inhibition of IL-6 binding to B-E8 by HAMA

Binding of IL-6 to B-E8 coated on microplates was measured by addition of 100 μl of 1 ng/ml biotinylated rIL-6 into each well for 2 h at room temperature. Inhibition of the ability of B-E8 to bind IL-6 was assessed by preincubating B-E8-coated plates with 100 μl of serum containing anti-B-E8 HAMA or not for 2 h before addition of biotinylated IL-6. Biotinylated IL-6 binding was measured as above. It was possible to take sera from three patients with renal carcinoma more than 23 days after the end of the B-E8 injections in which HAMA could be found but no B-E8 MoAb. Their capacity to inhibit the binding of B-E8 to IL-6 was compared with that of sera harvested

before treatment. Non-specific binding of biotinylated IL-6 was measured in wells containing 100 μl of 200 ng/ml rIL-6.

Inhibition of IL-6-dependent proliferation by B-E8 MoAb

The inhibitory property of B-E4 and B-E8 MoAb was tested on IL-6-dependent proliferation of the B9 cell line. Aliquots of 5000 B9 cells were cultured in 200 μl of RPMI 1640 medium supplemented with 5% fetal calf serum (FCS; Sepracor, Paris, France) and rIL-6 in 96-well microplates (Nunc) for 3 days. Proliferation was assayed by incubating the cells for another 8 h in the presence of tritiated thymidine (specific activity 25 Ci/mmol; CEA, Saclay, France), and counting the incorporated thymidine in a liquid scintillation analyser (TriCarb 1900 CA; Packard Instrument Company, Meriden, CT). One microgram of the B-E4 antibody was found to neutralize 3·3 ng of rIL-6; the B-E8 MoAb was about five-fold more potent, and 1 μg neutralized 18 ng of rIL-6. The inhibitory activity of sera containing B-E8 MoAb was measured by adding dilutions of the sera to the culture medium at the beginning of the culture and comparing it with cultures containing the same concentrations of the serum of the patient taken before the treatment.

Quantifiation of C-reactive protein

C-reactive protein (CRP) was determined by rate immunonephelometry using the Beckman array protein system (Beckman Instruments, Brea, CA).

RESULTS

Circulating B-E8 levels

Serum levels of B-E8 MoAb in a patient who received different doses of anti-IL-6 and never developed anti-B-E8 antibodies are illustrated in Fig.1a. When a constant amount of B-E8 was injected daily, the circulating concentration reached an equilibrium within 6 days and then levelled off, indicating a half-life of B-E8 MoAb of about 3 days. Increasing the doses injected resulted in a roughly proportional augmentation in B-E8 in the plasma. Due to its short half-life, B-E8 MoAb disappeared rapidly from the circulation when injections were interrupted.

Immunization against B-E8

Three of the six patients with MM and 6/6 of the patients with renal carcinoma became immunized against B-E8 (Table 2). Seven of the nine immunized patients (two with MM and five

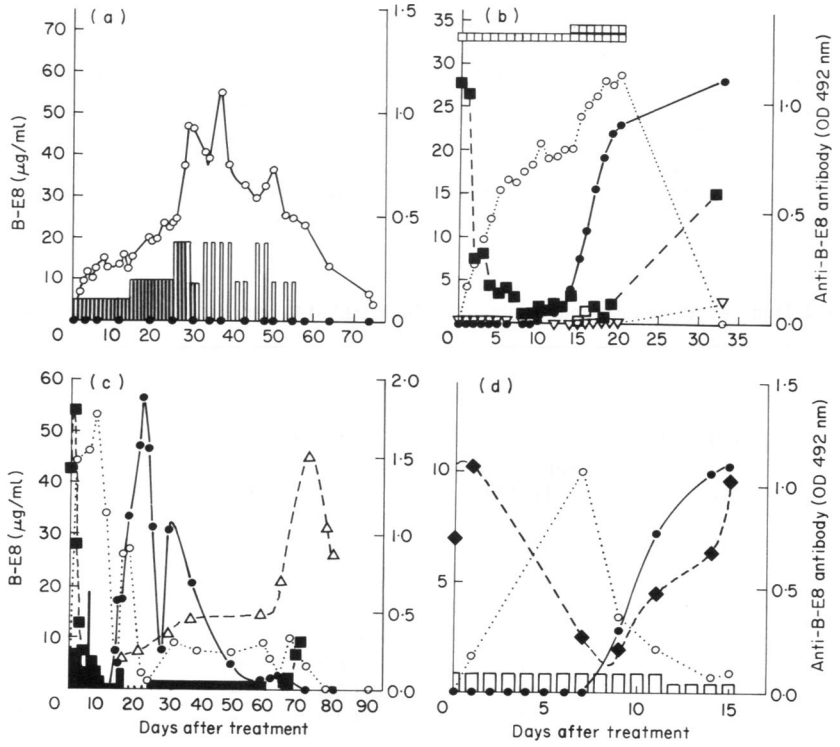

Fig. 1. Circulating levels of B-E8 MoAb and human anti-mouse immunoglobulin antibodies (HAMA) in treated patients. (a) Patient 6 (multiple myeloma (MM)) was injected with daily doses of 20, 40 and 80 mg of B-E8 MoAb (□). (b) Patient 7 (metastatic renal cell carcinoma (MRCC)) was treated daily for 14 days with 20 mg B-E8, then for 7 days with 40 mg (□). C-reactive protein (CRP) dropped and stabilized at 3–8 mg/l from 75 mg/*l* at the beginning of treatment and reached 41 mg/*l* again 12 days after the end of the treatment. (c) Patient 4 (MM) received a complex, long term treatment (■, [7]). HAMA IgM (not shown) appeared 24 h before the IgG; both IgG and IgM HAMA disappeared during the treatment. Antibodies against the antigenic contaminant of the B-E8 preparation which appeared at the same time as the HAMA levelled off; they were boosted after resuming the treatment at day 65. Note the well controlled CRP levels during treatment. (d) HAMA were seen at day 9 in patient 5 (MM) who was treated with 20 mg/day of B-E8 (□). Immediately, the circulating B-E8 dropped, the CRP increased (not shown) and the circulating plasmablasts which had decreased from 4% to 2% rose to values before treatment. □ B-E8 injections; ○, B-E8 in serum; ●, anti-B-E8 antibodies; △, anti-X antibodies; ▽, anti-IgG1 antibodies; ■, CRP; ◆, plasmablasts.

with MRCC) showed a similar type of immunization. In this group, antibodies against B-E8 arose in the plasma between days 7 and 15. In patient 7, shown as a representative example (Fig. 1b), antibodies became detectable at day 12. All patients produced IgG antibodies. Only four produced IgM; IgM appeared in the plasma 24 h earlier than the IgG in three patients, 24 h later in the fourth (Table 2). Antibodies reacted against B-E8 but not against other IgG1 MoAbs, and against neither B-E4 nor BL15. This was confirmed by inhibition experiments, where only B-E8, but none of the other control MoAbs, was able to inhibit the binding of human antibodies to B-E8 (data not shown). Clearly, circulating levels of B-E8 remained unaffected by the presence of these anti-B-E8 antibodies. Since we had no plasma samples from the days immediately following the end of the treatment, we could not study whether the half-life of B-E8 was decreased in cases of immunization. In one patient with MRCC, anti-B-E8 HAMA disappeared very rapidly, starting to decrease by the end of the B-E8 treatment. In another patient with MM (patient 4), anti-B-E8 antibodies disappeared during the treatment (Fig. 1c). We tested the patient's plasma against a less purified fraction of B-E8 precipitated by 40% ammonium sulphate and found a reactivity. We ran the ammonium-precipitated fraction on a Protein A column and used the effluent and eluted fractions for testing patient 4 plasma in ELISA in the same conditions as above. We found an activity against the effluent, and confirmed the absence of reactivity against the Protein A-eluted B-E8 MoAb; HAMA reactivity was probably directed against a non-immunoglobulin contaminant murine protein (called X in Fig. 1c). Interestingly, antibodies to this contaminant constantly arose during the treatment. We attempted to identify this contaminant, and found that it was also present in X63/Ag86553 ascites, but absent from therapeutic preparations of B-E8 obtained from *in vitro* cultures of B-E8 hybridoma, which showed that it was probably a murine protein copurified with B-E8 in the preparation used for therapy. We found no activity of patient 4 serum against purified murine albumin.

In the other two immunized patients a different course of immunization was observed. In patient 5 (Fig. 1d), the appearance of antibodies coincided with an extremely rapid disappearance of B-E8 from the circulation and, immediately, a dramatic increase of tumour plasmablasts in the blood. Antibodies against the other IgG1 MoAbs, were evidenced. IgM antibodies against an IgG2b MoAb were also observed in patient 8 (Table 1). Analysis of the HAMA specificity showed that inhibition of HAMA binding to B-E8 could be achieved only by B-E8, whereas the binding to another IgG1 (BL14) was inhibited by both B-E8 and BL14 (Fig. 2). Binding of HAMA to B-E8 should have been inhibited to some extent by class-matched BL14 MoAb; however, we repeated the experiments using dilutions of sera containing HAMA and always found the same result. CD8, BL15, an IgG2b MoAb, inhibited the fixation of human antibodies neither to B-E8 nor to BL14, indicating the presence of both anti-idiotypic and anti-isotypic antibodies. Maximum OD values measuring HAMA were of the same order in most of the patients (Table 2), therefore indicating that the proportion of HAMA could not be much higher in patients 5 and 8, nor could anti-isotypic antibodies be more concentrated than the anti-idiotypic, or *vice versa*.

Inhibitory properties of human anti-B-E8 antibodies

Several experiments were carried out to assay the ability of anti-B-E8 HAMA to inhibit the binding of IL-6 to B-E8. For this we used three serum samples from patients (all with MRCC) taken 15 days after interruption of the B-E8 injections which contained HAMA but no longer any detectable B-E8. Slight but

Table 2. Human anti-mouse immunoglobulin antibody (HAMA) response in the 12 patients studied

Patients	Pathology	Duration of treatment (days)	Appearance of HAMA	HAMA					
				Anti-BE8		Anti-BL14		Anti-BL15	
				IgG*	IgM	IgG	IgM	IgG	IgM
2	MM	13	–	–	–	–	–	–	–
3	MM	17	–	–	–	–	–	–	–
6	MM	55	–	–	–	–	–	–	–
1	MM	11	11	+(0·20)	–	–	–	–	–
4	MM	67	11	+(1·87)	+†	–	–	–	–
7	MRCC	21	14	+(1·20)	–	–	–	–	–
9	MRCC	21	12	+(1·37)	–	–	ND	–	ND
10	MRCC	21	7	+(0·72)	–	–	ND	–	ND
11	MRCC	21	15	+(0·69)	+†	–	–	–	–
12	MRCC	21	13	+(0·96)	–	–	–	–	–
5	MM	17	9	+(1·10)	+†	+	+	–	–
8	MRCC	21	9	+(1·20)	+‡	+	+	–	+

IgG and IgM anti-B-E8 (IgG1), anti-BL14 (IgG1) and anti-BL15 (IgG2b) antibodies were assayed by ELISA as in Patients and Methods.

* Figures in parentheses represent the maximum OD observed.

† IgM before IgG.

‡ IgM after IgG.

–, No HAMA detected; ND, not done.

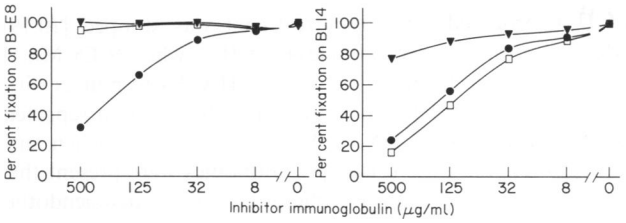

Fig. 2. Specificity of human anti-mouse immunoglobulin antibodies (HAMA) in patient 5. Inhibition of the binding to B-E8 and to BL14 (IgG1) of HAMA IgG present in the patient's plasma at day 14 of treatment. Various amounts of B-E8, BL14 or BL15 were added to the plasma before it was assayed for reaction against B-E8 or BL14. Note that only B-E8 MoAb was able to inhibit binding of HAMA to B-E8, whereas both BL14 and B-E8 could inhibit the binding to BL14. Inhibition by ●, IgG1, B-E8; □, IgG1, BL14; ▼, IgG2b, BL15.

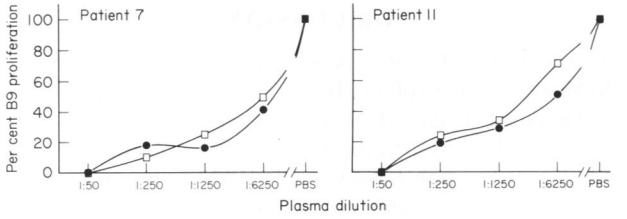

Fig. 3. Effect of the presence of human anti-mouse immunoglobulin antibodies (HAMA) on the inhibitory activity of B-E8 on IL-6. Inhibition of the proliferation (^{3}H-thymidine incorporation) of the IL-6-dependent B cells by B-E8 MoAb contained in various dilutions of the plasmas of two patients taken at day 10 of treatment, and at days 19–20 when HAMA were at maximum. Sera contained similar amounts of B-E8: patient 7 day 10, 21 μg/ml; patient 7 day 19, 29 μg/ml; patient 11 day 10, 21 μg/ml; patient 11 day 19, 28 μg/ml. Patient 7: ●, plasma day 10; □, plasma day 20. Patient 11: ●, plasma day 10; □, plasma day 19.

consistent inhibition of IL-6 binding to B-E8 was noticed in the three cases (Table 3).

To see whether this inhibition could account for a consequent inactivation of B-E8, we tested the ability of sera of patients containing both B-E8 MoAb and anti-B-E8 HAMA to inhibit the proliferation of the IL-6-dependent cell line B9; we tested plasmas containing similar amounts of B-E8 MoAb, and HAMA or not for comparison. Figure 3 shows the results obtained with sera of patients 7 and 11 taken at days 20 and 19, respectively, when the maximum of anti-B-E8 antibodies was found, in comparison with the sera of day 10 which contained the same amount of B-E8 but no anti-B-E8 antibodies. The ability of the B-E8 MoAb present in the plasma to inhibit the proliferation of B9 cells was not diminished by the presence of anti-B-E8 HAMA.

Table 3. Inhibition of fixation of biotinylated rIL-6 to B-E8 MoAb by patient's sera containing human anti-mouse immunoglobulin antibody (HAMA)

Serum	Hama (OD)	Biotinylated IL-6 binding in PBS	Biotinylated IL-6 binding in IL-6 (200 ng/ml)	Per cent inhibition
7 (D0)	–	0·89 ± 0·03	0·18 ± 0·01	
7 (D53)	+	0·69 ± 0·01	0·18 ± 0·01	28
9 (D0)	–	0·73 ± 0·01	0·15 ± 0·09	
9 (D44)	+	0·60 ± 0·02	0·15 ± 0·04	22
12 (D0)	–	0·75 ± 0·04	0·15 ± 0·01	
12 (D71)	+	0·61 ± 0.03	0·15 ± 0·09	23

Sera from patients 7 (D53), 9 (D44) and 12 (D71) were taken 32, 23 and 50 days, respectively, after the end of the 21-day treatment; they contained anti-B-E8 IgG antibodies but no longer any detectable B-E8 MoAb. Undiluted sera were added to B-E8-coated plates before addition of a tracer dose of 1 ng/ml biotinylated rIL-6. Non-specific binding of biotinylated rIL-6 was measured in wells containing 100 μl of 200 ng/ml unlabelled rIL-6. Inhibition of biotinylated rIL-6 binding by anti-B-E8 HAMA at day Dx was calculated in comparison with the binding in the presence of the patient's plasma taken before the treatment (D0) =

$$1 - \frac{\text{binding (D}x\text{)} - \text{binding (D}x\text{) with 200 ng/ml IL-6}}{\text{binding (D0)} - \text{binding (D0) with 200 ng/ml IL-6}}$$

Treatment in the presence of anti-B-E8 antibodies

Since anti-idiotypic antibodies to B-E8 MoAb seemed neither to inhibit B-E8 nor to induce its rapid elimination, we initiated a new series of five daily injections in a patient 15 days after the end of the first treatment. The patient still possessed anti-B-E8 HAMA and had recovered his initial high CRP level. Clinical tolerance was found to be as good as the first time. Three days after starting the new treatment, B-E8 was found in the plasma at levels similar to those seen the first time, and CRP had decreased (Fig. 4).

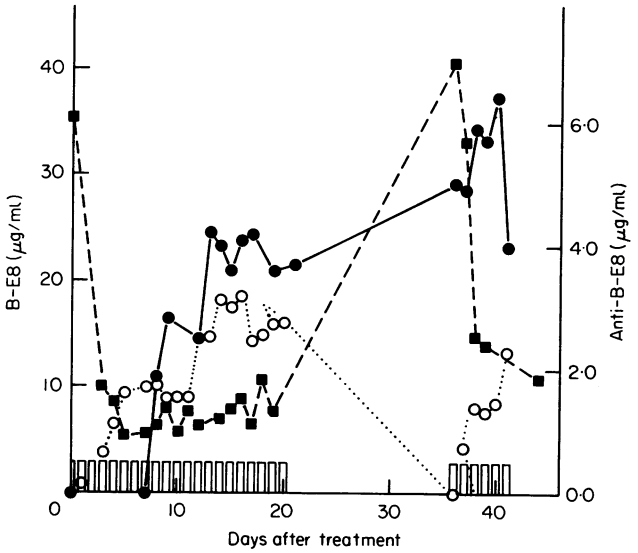

Fig. 4. Second treatment with B-E8 in a patient with circulating human anti-mouse immunoglobulin antibodies (HAMA). Patient 9 received five injections of 20 mg of B-E8 15 days after the end of a first series of 21 daily injections. Anti-B-E8 IgG antibodies were hardly increased. Kinetics of B-E8 appearance in the plasma and C-reactive protein (CRP) drop were similar to those observed at the beginning of the first period of treatment. CRP values ranged from 200 mg/l before treatment to 35–50 mg/l during the treatment. □, B-E8 injections; ○, B-E8 in serum; ●, IgG anti-B-E8 antibodies; ■, CRP.

DISCUSSION

Our results clearly show that immunization against murine MoAb recognizing soluble IL-6 is as frequent as that against MoAb directed against cellular structures [2]. Half of the patients with MM became immunized to anti-IL-6 MoAb, and all patients with MRCC made HAMA. It is known that the capacity of patients with MM to form antibodies to exogenous antigens is impaired [14]; no patients in this study were evaluated for their ability to make antibodies. Recently it was reported that five patients with rheumatoid arthritis had been treated with the same B-E8 MoAb; only two of them developed HAMA [15]. Several peculiarities of the immunization were noticed: all immunized patients synthesized IgG anti-B-E8 antibodies, but most of them did not produce IgM antibodies; in one case IgM anti-B-E8 antibodies appeared after the IgG. Immunization against B-E8 was sometimes transient, even during the MoAb treatment, as in patient 4. In that case, one can say that the phenomenon could not be due to non-specific anergy, since the patient was developing, at the same time, a consistent antibody response against a murine antigenic contaminant. Targeting of IL-6 could cause some specific anergy.

As was noticed in the response against CD3 and CD4 MoAb recognizing cellular antigens [4–6], HAMA to anti-IL-6 MoAb were restricted to two types of antibodies directed against isotypic or idiotypic epitopes. Although we never used $F(ab')_2$ fragments of MoAb, proofs of the presence of anti-idiotype antibodies seemed to be convincing. Anti-idiotype anti-B-E8 HAMA bound neither to several MoAbs of the same isotype from BALB/c mice, nor to another anti-IL-6 MoAb which recognized another epitope of IL-6. Anti-idiotype HAMA were the main cause of treatment inefficiency in transplanted patients treated for immunosuppression with CD3 MoAb [5], as well as in patients with cutaneous T cell lymphoma treated with CD5 MoAb [16]. Antigenic modulation on the surface of cellular targets [17,18], or clearance of the MoAb from the circulation in the presence of HAMA [5] accounted for the inhibitory effect. Although they were able to bind to the paratope of B-E8 (significant inhibition of the binding of biotinylated IL-6 to B-E8), anti-idiotypic HAMA were unable to inactivate a great proportion of the binding of B-E8 to IL-6, and did not decrease the inhibitory activity of the MoAb in the IL-6-dependent B9 assay. This contradiction might be explained by insufficient HAMA concentration in the circulation, although, judged on OD values, HAMA levels did not appear to be higher in patients 5 and 8, whose sera inhibited B-E8. In fact, when MoAbs are directed against soluble antigens, we seem to face a different situation. B-E8 MoAb did not clear faster from the circulation in patients immunized against idiotypic epitopes only, showing that soluble B-E8–anti-B-E8 complexes circulated in the plasma. We did not try to isolate and study these complexes, but the situation might be comparable to that encountered with IL-6–B-E8 complexes. We demonstrated earlier in the plasma of treated patients that such monomeric complexes, of 185 kD, dissociating at acid pH into functional B-E8 and IL-6, did circulate [19]. These complexes were still able to bind IL-6. Trapped in the monomeric complexes, IL-6 became unable to reach its target, as long as B-E8 was present [19], which accounted for the efficiency of the treatment. This also explains the apparent rise of IL-6 observed when the treatment was stopped [10,19], which was only due to the release of IL-6 when B-E8 MoAb had disappeared from the circulation. HAMA responses without any evident deleterious effect have already been reported, for instance against CD4 MoAb [20]. In contrast, when anti-idiotypic as well as anti-isotypic antibodies were present they caused the complexes to be rapidly removed (reticuloendothelial trapping?), leading to rapid inefficiency of the treatment, as was noticed in patients 5 and 8. It would be worth carrying out electrofocusing experiments to see whether the anti-B-E8 response is as oligoclonal as the one against CD3, OKT3 or CD4, OKT4 [6]. Owing to the narrow specificity of HAMA for B-E8 MoAb, we should expect a very restricted response.

According to the idiotypic theory, internal images of IL-6 might be found among anti-idiotypic anti-B-E8 HAMA. Observation of an elevated IL-6 activity in the plasma of one patient soon after interruption of the B-E8 injections [10] led us to investigate this point; in fact, we were unable to find any antibody of this type, and showed by gel filtration separation that the IL-6 activity was indeed IL-6 released from IL-6–B-E8 complexes [19].

Our results suggest the feasibility of efficient long term treatment as long as only anti-idiotypic (and perhaps only anti-isotypic) HAMA is produced. We tested this hypothesis by resuming treatment in a patient who had developed anti-idiotypic HAMA 15 days after the end of the first treatment, when he still had HAMA in his circulation; no evidence of bad tolerance was seen and, clearly, the rise in B-E8 in the plasma and the drop in CRP indicated an efficient anti-IL-6 treatment. However, we do not have experience of longer treatment, which might allow the production of neutralizing HAMA more frequently.

In conclusion, our results show that the type of HAMA response in patients treated by anti-IL-6 MoAb was similar to that already reported in patients receiving MoAb against cellular targets. However, consequences of the efficiency of the MoAb treatment were different. The apparent contradictions seem to be accounted for more by the mode of action of the target than by the properties peculiar to HAMA. As long as the response was restricted to one type of antibody (anti-idiotypic, or perhaps only anti-isotypic), the soluble complexes formed were not removed from the circulation, and the MoAbs were still able to bind IL-6 and to inhibit its traffic to its functional receptors. Appearance of HAMA against different epitopes led to rapid clearance of the MoAb.

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