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Phase I Evaluation of an Anti-Breast Carcinoma Monoclonal Antibody 260F9-Recombinant Ricin A Chain Immunoconjugate[1]

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ABSTRACT

Four women with metastatic breast cancer were treated with monoclonal antibody 260F9-recombinant ricin A chain, a ricin A chain immunoconjugate (IC) which targets a M_r 55,000 antigen expressed by human mammary carcinomas. Patients were treated by daily, 1-h i.v. injections for 6 to 8 consecutive days. Two patients were treated with 10 μg/kg daily and the two others were treated with 50 μg/kg daily. The trial was suspended after four patients had been treated because patients treated with a continuous infusion schedule with this IC had developed significant neurological toxicity at doses similar to those used in this study. The half-life of the IC showed a $t_{½}\alpha$ of approximately 1.8 h, a $t_{½}\beta$ of approximately 8.3 h, and a peak concentration of about 200 ng/ml, at the lower dose level, and showed a $t_{½}\alpha$ of approximately 2.5 h, $t_{½}\beta$ of about 10.4 h, and a peak concentration of 500 and 850 ng/ml for the two patients at the higher dose level. All four patients developed evidence of a human anticonjugate antibody response within 16 days of the onset of therapy. The treatment was associated with significant toxicity, manifested by a syndrome consisting of weight gain, edema, hypoalbuminemia, and dyspnea. Similar symptoms were observed in patients treated by continuous infusions of the IC. This clinical syndrome, seen at doses of IC which were insufficient to saturate antigen-expressing malignant tumor deposits in this trial, has been seen in other IC therapy trials and in clinical trials using the cytokine interleukin 2. To investigate a possible mechanism responsible for this toxicity, human monocytes were incubated with varying concentrations of IC. There was detectable binding of IC to human monocytes at IC concentrations which were achieved clinically in this trial. Furthermore, the binding appeared to be abrogated by preincubation of the monocytes with pooled human immunoglobulin, thus suggesting that binding occurs via Fcγ receptor-mediated mechanisms. Binding was not affected if different linkers between recombinant ricin A chain and the antibody were used or if a different antibody moiety was used in the IC preparation. Chemically linked dimers of MOPC-21 bound to human monocytes at least as well as the ICs; this binding was not abrogated by preincubation with pooled human immunoglobulin. Since the IC preparations used in this clinical trial contained small percentages of dimers and/or multimers, the clinical toxicity syndromes which we observed may be related to this series of observations. A more complete understanding of the relationship of this previously unreported mechanism of IC binding to human cells expressing Fcγ receptors with the clinical manifestations of the capillary leak syndrome will await production and testing of $F(ab')_2$ ICs or highly purified whole antibody IC preparations which contain only monomers. Further investigations into the mechanisms by which IC binding to Fcγ receptor-bearing cells may lead to disruption of endothelial cell integrity may provide important clues to the pathogenesis of the capillary leak syndrome seen with a variety of biological therapies.

INTRODUCTION

The availability of monoclonal antibody technology has made it possible to develop antibody-toxin conjugates that can deliver the toxin moiety to malignant cells in a highly specific manner. A variety of toxins have been tested; among the most potent of these is ricin. This toxin catalytically inhibits protein synthesis by binding to and cleaving the *N*-glycosidic bond of position A_{4324} in 28S RNA of 60S subunits of mammalian ribosomes (1–3). The native toxin is composed of two chains, A and B, linked by a disulfide bond. The B chain is responsible for binding to cell surfaces, while the A chain contains the enzymatic activity. ICs[3] containing purified A chains have been tested extensively in preclinical models and clinical trials (4–14).

The IgG1 murine monoclonal antibody 260F9 is specific for a M_r 55,000 antigen expressed by approximately 50% of breast cancer cells (15). This antibody has been conjugated to recombinant ricin A chain to form the immunoconjugate 260F9 MAb-rRA (16). Following extensive preclinical testing which confirmed the selectivity, biological activity, and acceptable toxicity profiles of the IC, Phase I evaluations of this compound were begun using two different administration schedules. This communication describes our observations of a daily injection schedule and focuses on the capillary leak syndrome seen with this IC. We show that this and other ICs bind to human cells that do not express the antigen identified by 260F9, via uptake by cellular Fcγ receptors.

MATERIALS AND METHODS

Clinical Study Design. Four patients with metastatic breast cancer were treated with this immunoconjugate between May and August 1987. Their clinical characteristics are summarized in Table 1. Patients 1 and 2 were assigned to receive 10 μg/kg daily of immunoconjugate, administered by a 1-h infusion for 8 consecutive days. Patient 3 was scheduled to receive 50 μg/kg daily by the same route for 8 consecutive days, while patient 4 was assigned to receive 50 μg/kg daily for 6 days based upon pharmacokinetic studies in the first three patients. The patients were hospitalized to receive all treatments in the clinical study unit at Fox Chase Cancer Center.

The patients were evaluated for toxicity on a daily basis while receiving treatment and at approximately weekly intervals thereafter. Unless specified otherwise, all toxicity is graded according to the recently developed National Cancer Institute common toxicity criteria.

IC Preparation. 260F9 MAb-rRA IC was prepared by the Cetus Corporation. Briefly, purified 260F9 MAb was linked to rRA through a linker containing a disulfide bond. Ricin A chain was produced in *Escherichia coli* K12 from the gene sequence of ricin A chain cloned from the complementary DNA of the castor bean. The final IC was purified free of unconjugated rRA and conjugates containing multimers of MAb and rRA; however, a small fraction of contaminating MAb-MAb-rRA persisted in the final product. Clinically used IC preparations contained a small concentration of dimers and multimers, less than 10% as assessed by nonreducing gel electrophoresis.

ICs 260F9 MAb-L1-rRA and 260F9-L2-rRA were prepared at Cetus using two thioether linkers, L1 and L2, to aid in determining the role

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[3] The abbreviations used are: IC, immunoconjugate; 260F9 MAb-rRA, murine monoclonal antibody 260F9-recombinant ricin A chain immunoconjugate; rRA, recombinant ricin A chain; SPDP, *N*-succinimidyl-3-(2-pyridylthio)propionate.

Table 1 *Patient characteristics*

Patient	Age (yr)	PS (%)[a]	Prior therapy[b]	Metastatic sites
1	35	90	Melph, 5-FUra, CTX, MTX, DXR, sternal XRT	Chest and abdominal wall Bone
2	71	90	5-FUra, MTX, VLB, CTX, DXR, MMC	SCN Bone
3	33	100	MTX, 5-FUra, Pred, Chlor	Lungs
4	54	80	MTX, MMC, TAM, CTX, VLB, 5-FUra, chest wall XRT	Chest wall, left SCN pleura, lungs

[a] PS, Karnofsky performance status; Melph, melphalan; 5-FUra, 5-fluorouracil; CTX, cyclophosphamide; MTX, methotrexate; DXR, doxorubicin; XRT, radiation therapy; VLB, vinblastine; MMC, mitomycin C; Pred, prednisone; Chlor, chlorambucil; Tam, tamoxifen; SCN, supraclavicular lymph node.
[b] Excluding surgery.

of the disulfide bond in the binding of the clinically tested IC to human monocytes.

MOPC-21 MAb-MAb ICs were produced at Cetus using SPDP linker technology. The final mixture was purified of free MAb and higher order multi-MAb conjugates by a column sizing step.

Pharmacokinetics and Anti-Conjugate Antibody Determinations. Samples were obtained at baseline; at 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 12.0, 18.0, and 24.0 h; and then daily prior to treatment. On the last day of therapy, blood was sampled prior to and just following dosing, and 24 h after this last injection. Serum levels of 260F9 MAb-rRA were determined using a double sandwich enzyme-linked immunosorbent assay. The sensitivity of this assay is less than 1.0 ng/ml. One component of the sandwich was directed at the rRA and the other at the murine MAb component; thus the assay determined the serum concentration of conjugates composed of rRA and murine MAb. Analysis was performed using a nonlinear, least squares fit of the data using a two compartment model, which fit the data better than a one compartment model. Data were weighted inversely and the fit program used was Simusolv (Dow Chemical Company). All serum sample times for each patient were incorporated into the data set used for the fitting of the presented parameters.

Anti-drug and anti-drug component antibody titers were determined using an enzyme-linked immunosorbent assay. Briefly, the drug or one of its components (260F9 MAb or rRA) was coated on plates. Appropriate dilutions of patient serum were incubated in the plate, unbound material was washed free, and the plate was developed with peroxidase anti-human IgG or peroxidase anti-human IgM. The isotype-matched murine antibody 520C9 was used as a plate coat to distinguish between responses directed at the binding specificity of 260F9 (antiidiotype) and those directed at the constant regions of murine IgG1 antibodies.

Cell Phenotype Studies. A variety of antibodies and ICs were used for *in vitro* analysis of binding to monocytes. Pooled human IgG, MOPC-21, and UPC-10 myeloma proteins were obtained from Sigma Chemical Co., St. Louis, MO. Monoclonal antibody 260F9 was generously provided by Dr. David Ring (Cetus Corporation). 260F9 MAb-rRA, MOPC-21 MAb-rRA, 260F9 MAb-L1-rRA, and 260F9 MAb-L2-rRA were all prepared by Cetus Corporation. $F(ab')_2$ fragments of goat anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate were purchased from Cappel, Malvern, PA.

Peripheral blood was obtained from normal donors and peripheral blood mononuclear cells were purified on Ficoll-Isopaque gradients, as described (17). In some experiments purified monocytes were obtained by reversible binding to gelatin and autologous plasma-coated plastic flasks, as described (18). To evaluate binding of various proteins to monocytes, purified monocytes or peripheral blood mononuclear cell suspensions were incubated with various concentrations of the binding proteins for 30 min at 4°C. Cells were washed and $F(ab')_2$ fragments of goat anti-mouse IgG labeled with fluorescein isothiocyanate were added. Following another 30-min incubation at 4°C the cells were washed and fluorescence was evaluated by flow cytometry using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Gates were set on monocytes by forward and side scatter criteria; greater than 95% of the cells in the monocyte gates expressed Leu-M3 (data not shown). Results are expressed as percentage of cells with more fluorescent intensity than 95–98% of the negative control cell population. Statistics were performed using paired *t* test analysis, as described (19).

RESULTS

Clinical Course. The courses of treatment are summarized in Table 2. Patients 1 and 2 received their planned therapy at full doses for all 8 days. Patient 3 required a 1-day treatment break and 50% dosage reductions thereafter, so that she received therapy for 7 full days, with 62.5% of her planned dose actually administered. Patient 4 received full doses of a 6-day planned treatment course. The shorter treatment course was undertaken because of the demonstration of anticonjugate antibody responses observed in the previous patients (see below).

One patient, #3, had a clinical response, with the disappearance of her sole site of disease, a 1-cm^3 lung nodule identified by chest computer-assisted tomographic scan. However, she had other lung abnormalities, also seen on computer-assisted tomographic scan, of uncertain disease significance, which did not change with therapy. She had recurrence of her tumor at a chest wall/skin site 3.5 months after the initiation of her therapy.

A number of toxicities were observed with this therapy, even at doses which were too low to yield detectable binding of the immunoconjugate to the target antigen of 260F9 (Table 3). Malaise and other constitutional symptoms such as fever or myalgias were commonly seen but were not dose limiting. While all patients experienced performance status deterioration, only patient 3 declined significantly. Peripheral blood monocyte counts did not change with therapy. Anemia was universal and could not be totally accounted for by multiple phlebotomies. Weight gain averaging 4.2 kg was observed as was edema, hypoalbuminemia, hypoproteinemia, and eosinophilia. Patient

Table 2 *Treatment summary*

Patient	Assigned daily dose (μg/kg)	Planned total dose (mg)	Duration of therapy (days)		Total dose received (mg)
			Planned	Actual	
1	10	6.8	8	8	6.8
2	10	5.5	8	8	5.5
3	50	20.7	8	7[a]	13.0
4	50	17.7	6	6	17.7

[a] This patient received full doses for 3 days. Treatment was held for 1 day and then resumed at 50% doses for the remaining 4 days.

Table 3 *Adverse events during therapy*

Toxicity	Patient			
	1	2	3	4
Maximum Karnofsky performance status drop (%)	10	10	40	10
Highest fever (grade)	0	0	1	2
Most wt gain (kg)	5	5	5.2	1.7
Hypotension (grade)	0	0	3	0
Tachycardia (grade)	0	0	0	0
Edema (grade)	0	1	2	2
Largest hemoglobin drop (g/100 ml)	3.1	2.4	1.6	1.7
Eosinophilia (%)	4	10	8	3
Serum albumin (% of baseline)	69	77	60	79
Serum total protein (% of baseline)	71	83	69	86
Rash (grade)	0	0	1	0
Dyspnea (grade)	0	2[a]	0	0
Neurological (grade)	0	0	1[b]	1[c]
Nausea, vomiting (grade)	0	0	2	1
Flu-like symptoms (grade)	0	0	1	1

[a] Delayed onset of dyspnea in absence of pulmonary tumor, edema, or further weight gain.
[b] Delayed onset of fingertip paresthesias (see text).
[c] Left arm weakness and diminished sensation. Tumor in brachial plexus found on magnetic resonance imaging scan.

3 experienced grade 3 orthostatic hypotension which was associated with weight gain, edema, hypoalbuminemia, and hypoproteinemia. Her clinical picture closely matched that of the capillary leak syndrome which has been observed in patients receiving high dose interleukin 2 therapy (20, 21). In addition, this patient experienced a grade 2 nonpruritic rash on her trunk and extremities following the last dose of immunoconjugate.

Three of the four patients experienced delayed symptoms which were possibly attributable to the immunoconjugate therapy. Patient 2 experienced grade 2 dyspnea 6 days after the conclusion of therapy in the absence of any demonstrated pulmonary tumor, edema, or weight gain. She was thought to have congestive heart failure by her local treating physicians, although this was never documented by noninvasive or invasive studies. Patient 3 developed the delayed onset of fingertip paresthesias in the arm contralateral to her prior mastectomy incision. These symptoms resolved completely within 6 months. She has since developed evidence of local skin recurrence but has had no evidence of axillary or brachial plexus involvement. Finally, patient 4 experienced progressive left arm weakness and diminished sensation in that arm following therapy. However, extensive tumor was found in the left brachial plexus upon magnetic resonance imaging scanning.

Pharmacokinetics and Anti-Drug Antibodies. Pharmacokinetics are displayed in Table 4A. The two patients treated with 10 μg/kg/day developed peak serum concentrations of approximately 200 ng/ml. $t_{\nu_1}\alpha$ was approximately 1.8 h and $t_{\nu_2}\beta$ was approximately 8.3 h for these two patients. For the two patients treated with doses of 50 μg/kg/day, $t_{\nu_1}\alpha$ was approximately 2.5 h, $t_{\nu_2}\beta$ was was approximately 10.4 h and the peak serum levels were approximately 500 and 850 ng/ml, respectively. Trough levels of IC were noted to decline in patient 1 during the last 2 days of therapy (from 25 to 10 ng/ml). The role of anti-drug antibodies in mediating this decrease is unclear. Peak serum levels did not change in this patient from days 1 to 8 (264 to 248 ng/ml).

As shown in Table 4B, all four patients developed anticonjugate antibody titers, which in all cases exceeded the titers to either the antibody or ricin A chain components of the conjugate. Significant IgG titers were noted against 260F9 antibody;

Table 4 *Pharmacokinetics: one and two compartment fit models*

A. Pharmacokinetics

	Patient 1	Patient 2	Patient 3	Patient 4
Daily dose (μg/kg) (1-h infusion)	10	10	50	50
Peak serum concentration (ng/ml)	260	170	850	500
Half-life (h)	4.9 ± 1.3	4.4 ± 1.7	5.0 ± 1.1	7.1 ± 1.9
Volume of distribution of central compartment (ml/kg)	39.1 ± 0.2	69.1 ± 0.5	63.7 ± 0.1	90.7 ± 0.2
$t_{\nu_1}\alpha$ (h)	1.1 ± 0.04	2.6 ± 0.3	3.0 ± 0.1	2.0 ± 0.04
$t_{\nu_2}\beta$ (h)	7.9 ± 0.1	8.7 ± 1.3	10.9 ± 0.5	9.9 ± 0.1
Amplitude to $t_{\nu_2}\beta$	0.49 ± 0.01	0.32 ± 0.09	0.15 ± 0.01	0.42 ± 0.01
Area under curve (ng/ml-h)	1620	960	4808	4237
Clearance (ml/kg-h)	6.2	10.4	10.4	11.8
V_{ss} (ml/kg)	62.7	95.5	91.9	139.0

B. Human anti-conjugate antibody responses

		IgG (peak titer)				IgM (peak titer)			
Patient	First day of rise	Conjugate	260F9	rRA	520C9	Conjugate	260F9	rRA	520C9
1	8	80	40	35	3	15	7	<1	4
2	16	20	5	5	5	4	2	<1	4
3	10	300	40	30	30	30	20	4	20
4	15	10	9	1	7	2	<1	<1	<1

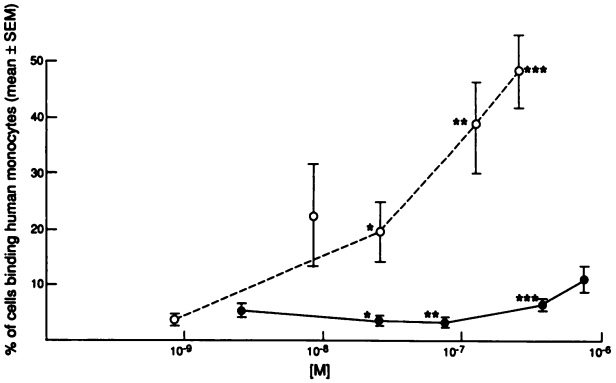

Fig. 1. The binding of the IgG1 murine antibody 260F9 (●) and 260F9 MAb-rRA IC (○) to human monocytes was assessed by flow cytometry as described in "Materials and Methods." At protein concentrations exceeding 1×10^{-8} M, the IC bound significantly more than did native antibody. *, $P < 0.01$; **, $P < 0.05$; ***, $P < 0.001$.

in patient 1, this appeared to be largely an antiidiotype response since the human anti-mouse antibody response against the IgG1 murine antibody 520C9 was substantially less than that of 260F9. The development of an antiidiotype response distinguishable from a human anti-mouse antibody response was not clear in the other patients tested. In general, the IgG peak titers substantially exceeded the IgM peak titers. In all patients, a human anticonjugate response was noted with 16 days of the onset of treatment and as early as 8 days following the onset of therapy in patients 1 and 3.

Patient Tumor Studies. In patient 1 an abdominal wall metastasis was biopsied and found to demonstrate the antigen recognized by antibody 260F9. A chest wall metastasis was biopsied at the conclusion of 8 days of treatment and analyzed for antigen expression and persistence of the immunoconjugate. While the malignant cells in this lesion expressed antigen, as measured by the method of immunoperoxidase staining of frozen lesion sections with 260F9 antibody, no persistent immunoconjugate was detectable in the lesion (data not shown). This finding is not unexpected since higher doses have been required to achieve saturation of tumor binding sites in clinical trials with other antibodies (22).

Toxicity Mechanism Studies. The identification of significant capillary leak syndrome toxicities in association with immunoconjugate therapy suggested the possibility that the immunoconjugate was delivering recombinant ricin A chain to unintended targets and that this delivery might be inducing the toxic syndrome. We chose to investigate the possibility that the addition of the ricin A chain had altered the structure of the IgG1 murine antibody 260F9 so that the modified protein was capable of binding to human Fcγ receptors. To test this, human monocytes were incubated *in vitro* with varying concentrations of immunoconjugate or with 260F9. As shown in Fig. 1, the native antibody did not bind to monocytes over a broad range of concentrations. In contrast, the immunoconjugate exhibited a concentration-dependent increase in binding so that detectable binding was noted at concentrations as low as 10^{-8} M. It should be noted that the peak serum IC concentrations even at the lower dose level (Table 4A) were associated with substantial binding to monocytes *in vitro*. This enhanced binding was observed not only when the monocytes were incubated with these proteins in small volumes for immunophenotypic analyses but also when the monocytes were incubated in tissue culture media at 1×10^6 cells/ml in the appropriate concentrations of immunoconjugate (data not shown).

To investigate the mechanisms by which this binding may occur, monocytes were incubated with a variety of ICs and other proteins as shown in Table 5. To evaluate the role of the 260F9-binding portion on monocyte binding, monocytes were exposed to an IC identical to 260F9 MAb-rRA except that the antibody portion of the IC was the MOPC-21 myeloma protein. To evaluate the possibility that the specific linker used to conjugate the antibody to ricin A chain was responsible for the observed results (through either disulfide exchange or another linker-specific mechanism), two ricin A chain 260F9 ICs prepared using different thioether linker technologies, designated 260F9 MAb-L1-rRA and 260F9 MAb-L2-rRA, were tested as well. The myeloma protein UPC-10, an IgG2a murine protein which interacts with the Fcγ receptor expressed by human monocytes, was tested as well. Finally, the ability of preincubation with 50 μg/ml pooled human IgG to inhibit IC binding via saturation of monocyte Fcγ receptors was evaluated as well. As shown by the representative experiment depicted on Table 5, all of the ICs bound well to monocytes. Furthermore, the binding of each IC was inhibited to control levels when the monocytes were preincubated with human immunoglobulin. The ICs did not bind to lymphocytes. To further evaluate the Fcγ receptor-mediated uptake of the immunoconjugate, a number of experiments were performed in which human monocytes were preincubated with human immunoglobulin prior to the addition of the IC. As shown in Fig. 2, preincubation with pooled human IgG clearly suppressed binding of the IC to human monocytes.

The clinically used IC preparations contained less than 10% dimers and multimers by nonreducing gel electrophoresis. To further investigate the mechanisms by which this IC may bind to the Fcγ receptor expressed by human monocytes, the ability of chemically constructed SPDP-linked dimers of MOPC-21 to bind to human monocytes was investigated. Fig. 3 demonstrates the results of a series of experiments in which monocytes were incubated with either MOPC-21, 260F9 MAb-rRA, or MOPC-21 dimers at varying concentrations. As expected, the immunoconjugate bound to human monocytes; this binding was inhibited by preincubation with human immunoglobulin. The MOPC-21 dimer bound to monocytes as least as well as the immunoconjugate. Furthermore, this binding was only partially reversed by preincubation with human immunoglobulin. Since the preparation used may have contained higher multimers of MOPC-21, it is possible that these immunoglobulin aggregates possessed substantially higher affinity for Fcγ receptors than did the dimers. These data suggest one possible explanation for the binding of this IC to human Fcγ receptor-bearing cells.

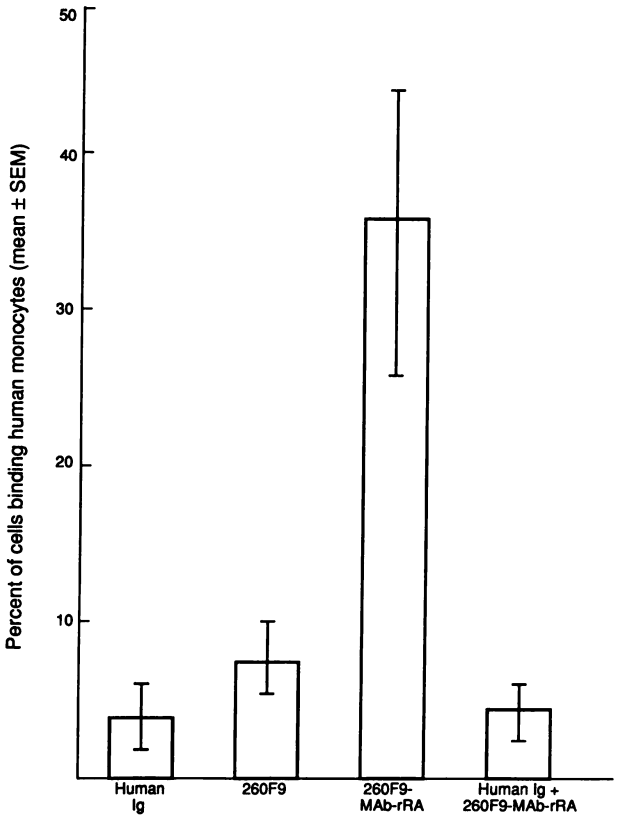

Fig. 2. Purified human monocytes were incubated with 10^{-8} M 260F9 or 260F9 MAb-rRA as described in the text. Cells were preincubated with either medium or 50 μg/ml of pooled human immunoglobulin (*Human Ig*) for 30–45 min. These conditions saturate human monocyte Fcγ receptors (data not shown). The binding of human immunoglobulin to monocytes could not be detected in this assay since $F(ab')_2$ fragments of goat anti-mouse immunoglobulin were used to detect the presence of adhered immunoglobulin.

DISCUSSION

This dose escalation phase I clinical trial of bolus IC administration was suspended after four patients had been accrued because patients treated by continuous infusion of the IC in another phase I trial developed severe and unexpected neurologic toxicity.[4] No serious neurotoxicity was observed with the bolus schedule, and we speculate that the continuous infusion schedule may have resulted in an increased area under the curve, permitting observation of the neurotoxicity at equivalent daily IC doses. We found two additional problems which may limit the likelihood of reaching therapeutically useful dosages of this IC. The first, not unexpectedly, is the development of anticonjugate antibodies. This response was seen as early as 8 days following the onset of therapy in our series. This response does not seem to be trivial in that patient 3 experienced a rash and in patient 1, who developed anticonjugate antibodies by day 8, peak serum levels of IC decreased late in treatment. The second problem was the toxic syndrome consisting of edema, hypoalbuminemia, weight gain, and, occasionally, congestive heart failure or pulmonary edema. This toxicity syndrome was not related to the schedule of administration of 260F9 MAb-rRA because similar toxicity was noted in patients treated with a continuous infusion schedule at doses of either 50 or 100 μg/kg daily for 8 days.

This symptom complex has been reported in clinical trials using other ICs incorporating the ricin A chain. Spitler *et al.*

Table 5 *Ricin A chain immunoconjugates bind to human monocytes via Fcγ*

	Preincubation with human immunoglobulin			
	Monocytes (% positive)		Lymphocytes (% positive)	
Tested protein (25 μg/ml)	No	Yes	No	Yes
Human immunoglobulin	9.7	—[a]	8.1	—
260F9	9.3	—	10.9	—
MOPC-21	3.6	—	10.4	—
UPC-10	91.4	—	12.0	—
260F9 MAb-rRA	48.2	7.4	12.9	8.4
MOPC-21-MAb-rRA	42.3	8.1	12.0	8.2
260F9-L1-rRA	29.0	6.4	11.6	8.1
260F9-L2-rRA	64.4	5.7	8.2	8.5

[a] —, not done.

[4] B. J. Gould, M. J. Borowitz, P. W. Carter, J. Moore, E. Groves, D. Anthony, L. M. Weiner, and A. Frankel. A phase I study of a continuous infusion anti-breast cancer immunotoxin: observation of a targeted toxicity not predicted by animal studies, submitted for publication.

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