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## PURE RED-CELL APLASIA AND ANTIERYTHROPOIETIN ANTIBODIES IN PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN

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### ABSTRACT

**Background** Within a period of three years, we identified 13 patients in whom pure red-cell aplasia developed during treatment with recombinant human erythropoietin (epoetin). We investigated whether there was an immunologic basis for the anemia in these patients.

**Methods** Serum samples from the 13 patients with pure red-cell aplasia were tested for neutralizing antibodies that could inhibit erythroid-colony formation by normal bone marrow cells in vitro. The presence of antierythropoietin antibodies was identified by means of binding assays with the use of radiolabeled intact, deglycosylated, or denatured epoetin.

**Results** Serum from all 13 patients blocked the formation of erythroid colonies by normal bone marrow cells. The inhibition was reversed by epoetin. Antibodies from 12 of the 13 patients bound only conformational epitopes in the protein moiety of epoetin; serum from the remaining patient bound to both conformational and linear epitopes in erythropoietin. In all the patients, the antibody titer slowly decreased after the discontinuation of treatment with epoetin.

**Conclusions** Neutralizing antierythropoietin antibodies and pure red-cell aplasia can develop in patients with the anemia of chronic renal failure during treatment with epoetin. (N Engl J Med 2002;346:469-75.)

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THE production of erythrocytes requires the hormone erythropoietin,<sup>1</sup> which in adults is produced mainly by the kidney. A lack of erythropoietin is the reason for the development of anemia in chronic renal failure.<sup>2</sup> The hormone is not absent in chronic renal failure, however, because the liver and partially functioning kidneys produce enough erythropoietin to maintain a low

level of erythropoiesis, as evidenced by the presence of erythroblasts in the bone marrow and reticulocytes in the blood.

The gene for human erythropoietin was cloned in 1985,<sup>3,4</sup> and recombinant human erythropoietin (epoetin) was approved for marketing in France in 1988 for the treatment of anemia in patients undergoing dialysis for chronic renal failure. Endogenous erythropoietin is a heavily glycosylated protein, and glycosylation is essential for its biologic activity. Endogenous erythropoietin and epoetin have different patterns of glycosylation, which involve primarily the sialic acid composition of oligosaccharide groups.<sup>5</sup> Epoetin alfa (Johnson & Johnson, Manati, Puerto Rico) and epoetin beta (Roche, Mannheim, Germany) are produced by recombinant methods in Chinese-hamster-ovary cells. They have slight differences in glycosylation; epoetin alfa has more sialic acid residues than epoetin beta.<sup>6</sup>

Since the introduction of epoetin into clinical practice, only three cases in which antierythropoietin antibodies developed after the administration of epoetin have been reported.<sup>7-9</sup> We studied 13 patients with chronic renal failure in whom severe transfusion-dependent anemia developed after an initial hematologic response to epoetin. In all 13 patients, the ane-

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mia was due to pure red-cell aplasia in association with neutralizing antierythropoietin antibodies.

## METHODS

### Bone Marrow Cultures

Otherwise normal patients undergoing hip-replacement surgery gave written informed consent for the collection of bone marrow cells. Erythroid and granulocytic cultures were established as previously described<sup>10</sup> with use of either normal pooled serum from 10 healthy volunteers (the control group) or each patient's serum at a final concentration of 20 percent. Erythroid and granulocytic colonies were assessed on days 7 and 14, respectively.

### Binding of <sup>125</sup>I-Labeled Epoetin

Highly purified epoetin was labeled with iodine-125 as previously described, with specific activities ranging from  $2.5 \times 10^7$  to  $5 \times 10^7$  counts per minute (cpm) per microgram.<sup>11</sup> Approximately 100,000 cpm of <sup>125</sup>I-labeled epoetin in 200  $\mu$ l of Tween bovine serum albumin in TRIS-buffered saline (consisting of 10 mM TRIS-hydrochloric acid, pH 7.4; 150 mM sodium chloride; 0.02 percent sodium azide; 0.1 percent bovine serum albumin; and 0.1 percent Tween 20) was incubated overnight at 4°C with different concentrations of patient serum or 20  $\mu$ l of control serum. Protein G Sepharose (50  $\mu$ l) (Pharmacia, Uppsala, Sweden) was added, and the tubes were incubated for another hour with continuous stirring. Then, 2 ml TRIS-buffered saline-Tween bovine serum albumin was added, and the tubes were centrifuged for 15 minutes at  $1500 \times g$ . The resulting pellets were washed twice, and the radioactivity was counted. This sensitive method can detect antibodies able to bind 200 mU of erythropoietin per milliliter of serum.<sup>10</sup>

### Deglycosylation Studies

Deglycosylation of epoetin was performed as previously described.<sup>12</sup> <sup>125</sup>I-labeled epoetin was diluted with 200  $\mu$ l of 50 mM sodium phosphate buffer (pH 5.0) containing 0.1 percent non-ethyleneglycol octylphenyl ether (NP40) and 0.02 percent sodium azide and sequentially deglycosylated by incubation for 1 hour at 37°C with *Arthrobacter ureafaciens* neuraminidase and for 18 hours with a mixture of O-glycosidase, endoglycosidase F, and N-glycosidase F (all from Roche, Mannheim, Germany). The efficiency of deglycosylation was monitored with the use of polyacrylamide-gel electrophoresis, and the extent of deglycosylation of unlabeled erythropoietin was confirmed by mass spectrometry.

### Denaturation of Epoetin

Deglycosylated, <sup>125</sup>I-labeled epoetin was denatured by boiling in 0.1 percent sodium dodecyl sulfate and 50 mM dithiothreitol in phosphate-buffered saline. The solution was boiled for five minutes before 250 mM iodoacetamide was added, and the solution was then incubated for one hour at 20°C in the dark. NP40 was added to achieve a final concentration of 1 percent, and the solution was diluted 1:1000 with TRIS-buffered saline before use. In control experiments, epoetin was added after dilution of the incubation medium containing sodium dodecyl sulfate, dithiothreitol, NP40, and iodoacetamide; these experiments showed that the final concentrations of these compounds did not affect the binding of antibody to epoetin.

## RESULTS

### Patients

The 13 patients we studied were identified during standard treatment of anemia due to chronic renal failure from May 1998 to November 2000. The clinical features of the 13 patients were similar (Table 1).

Twelve patients were receiving treatment in France and one was being treated in the United Kingdom. Eleven patients were undergoing hemodialysis, one was undergoing peritoneal dialysis, and one was not undergoing dialysis. All patients had been treated with epoetin by the subcutaneous route, and severe anemia that was resistant to epoetin had developed in all after 3 to 67 months of treatment. Twelve patients received epoetin alfa in the last few months before their disease became refractory to treatment (Table 1); one patient (Patient 3) was treated exclusively with epoetin beta, and her anemia also became refractory to treatment. The diagnosis of pure red-cell aplasia was based on the absence of erythroid cells in the bone marrow in 12 patients and the absence of circulating reticulocytes in 1 patient (Patient 11).

Thoracic and abdominal computed tomographic scans showed no evidence of thymoma, lymphoma, or solid tumor. The results of serologic tests for parvovirus B19, human immunodeficiency virus, Epstein-Barr virus, hepatitis viruses, and cytomegalovirus were negative. In serum samples collected at the time of the diagnosis of pure red-cell aplasia, serum erythropoietin (measured by an enzyme-linked immunosorbent assay with a commercial kit [R and D Systems Europe, Abington, United Kingdom]) was undetectable in 10 patients and within the normal range in 3 patients. This finding was surprising, because serum erythropoietin levels are usually very high in patients with pure red-cell aplasia.<sup>13</sup> Because antierythropoietin antibodies in serum can interfere with erythropoietin measurements by forming complexes with erythropoietin molecules, serum samples from these patients were tested for the presence of antierythropoietin antibodies.

Therapy with epoetin was discontinued when the presence of antierythropoietin antibodies was confirmed. As of September 2001, 6 of the 13 patients (Patients 2, 3, 6, 7, 8, and 9) had recovered some erythropoietic function after receiving immunosuppressive therapy or a renal allograft (Table 2). Two other patients (Patients 1 and 4), after a follow-up of more than two years, remain transfusion-dependent despite immunosuppressive treatment. Another patient (Patient 5) did not receive immunosuppressive treatment and remains transfusion-dependent 34 months after the onset of anemia. The follow-up period for the four remaining patients (Patients 10, 11, 12, and 13) was too short for conclusions to be drawn regarding their clinical course.

### Bone Marrow Cultures

We evaluated the ability of serum from epoetin-treated patients to inhibit the proliferation of erythroid progenitor cells by using cultured bone marrow cells from healthy donors. All 13 samples inhibited

TABLE 1. CHARACTERISTICS OF THE 13 PATIENTS.

PATIENT No.	AGE	RENAL DISEASE	DATE HEMODIALYSIS BEGUN	DATE SC EPOETIN THERAPY BEGUN*	INTERVAL FROM START OF THERAPY TO REFRACTORY ANEMIA	DATE OF DIAGNOSIS OF RED-CELL APLASIA BY BONE MARROW EXAMINATION
	yr				mo	
1†	71	Glomerulonephropathy	12/93	12/93, epoetin beta 4/97, epoetin alfa	53	2/99
2	22	Renal agenesis	6/98	7/98, epoetin alfa	3	2/99
3	40	IgA nephropathy	2/98	3/98, epoetin beta	7	2/99
4	64	Nephroangiosclerosis	11/98	2/99, epoetin alfa	4	8/99
5	68	Extramembranous glomerulonephritis	10/90	2/93, epoetin alfa‡	67	1/99
6§	29	IgA nephropathy	Not given	1/99, epoetin alfa 5/99, epoetin beta	5	7/99
7	44	Membranoproliferative nephropathy	3/98	11/98, epoetin alfa	10	11/99
8	44	Vascular nephropathy	3/99	6/99, epoetin alfa	3	10/99
9	72	Chronic pyelonephritis	1/99	10/98, epoetin alfa	12	10/99
10	71	Undetermined	6/00	11/99, epoetin alfa	4	3/00
11	85	Nephroangiosclerosis	3/99	3/00, epoetin alfa	7	10/00¶
12	55	Vascular nephropathy	3/98	6/00, epoetin alfa	5	2/01
13†	53	Undetermined	12/97	12/97, epoetin beta 12/98, epoetin alfa	32	11/00

\*SC denotes subcutaneous.

†Patients 1 and 13 first received epoetin beta, which was replaced by epoetin alfa at the indicated times.

‡Patient 5 received intravenous epoetin from February 1993 until June 1998, when subcutaneous therapy was begun.

§Patient 6 first received epoetin alfa, which was replaced by epoetin beta.

¶Bone marrow examination was not performed in Patient 11, who presented with a profound anemia without reticulocytes.

||Patient 12 underwent peritoneal dialysis.

the growth of erythroid progenitor cells but did not modify the formation of granulocytic colonies (Table 3). Tests performed on 12 of 13 serum samples showed that the addition of epoetin to the culture reversed the inhibitory effects of patients' serum on erythroid-colony formation (Fig. 1 and Table 3). Using serum from Patient 1, we found that inhibition of erythroid-colony formation was due to IgG. Removal of the IgG fraction of this serum with the use of immobilized G protein abolished its inhibitory effect, whereas IgG purified from the patient's serum inhibited erythroid-colony formation (Fig. 1). In contrast, IgG purified from control serum did not inhibit erythroid-colony formation. These results strongly suggested that the patients' serum contained neutralizing antierythropoietin antibodies. We therefore determined whether antierythropoietin antibodies were present in serum samples collected at the time of the diagnosis of pure red-cell aplasia.

#### Binding of <sup>125</sup>I-Labeled Epoetin

To test for the presence of antierythropoietin antibodies, we incubated increasing concentrations of

serum (1 to 20  $\mu$ l of serum per 200  $\mu$ l of incubation medium) with <sup>125</sup>I-labeled epoetin and separated immune complexes using protein G Sepharose. The results for Patient 1 are shown in Figure 2. Using the amount of serum required to bind 50 percent of the radioactivity in the presence of immobilized G protein, we estimated that 1 ml of this patient's serum was able to bind approximately 40 U of epoetin (Table 3). This value is in good agreement with the neutralization capacity estimated from results obtained with normal progenitor cells (Fig. 1). Serum from all 13 patients was found to bind epoetin (Table 3). In contrast, normal serum did not bind <sup>125</sup>I-labeled epoetin. As controls, serum samples from patients treated with epoetin who did not have pure red-cell aplasia were tested and found to be negative.

#### Characterization of Antierythropoietin Antibodies

Scatchard analysis<sup>14</sup> of the binding of antibodies in serum from Patient 1 to epoetin yielded an apparently straight line (Fig. 3), suggesting the presence of homogeneous binding sites, although the presence of some heterogeneity in the binding antibod-

TABLE 2. TREATMENT AND OUTCOME OF PURE RED-CELL APLASIA.\*

PATIENT No.	DATE OF ONSET OF ANEMIA	FOLLOW-UP (MO)	TREATMENT	OUTCOME
1	5/98	38	Immune globulin	Decrease in antibody titers; no decrease in transfusion requirements
2	10/98	33	Immune globulin, plasmapheresis, and corticosteroids	Disappearance of antibodies; no transfusion given since September 1999; hemoglobin level stable (10 g/dl)
3	10/98	33	Immune globulin and corticosteroids, followed by kidney transplantation in November 2000	No decrease in transfusion requirements until kidney transplantation; hemoglobin level then stable (11 g/dl) without transfusions
4	6/99	24	Corticosteroids	Progressive decrease in antibody titers; no decrease in transfusion requirements
5	9/98	34	No treatment	Progressive decrease in antibody titers; no decrease in transfusion requirements
6	6/99	24	Corticosteroids and cyclosporine	Disappearance of antibodies; no transfusion given since October 1999; hemoglobin level stable (13 g/dl)
7	9/99	22	Corticosteroids	Progressive decrease in antibody titers; no transfusion since June 2000; hemoglobin level stable (12 g/dl)
8	9/99	22	Corticosteroids	Progressive decrease in antibody titers; no transfusion since February 2000; hemoglobin level stable (8.5 g/dl)
9	10/99	21	Cyclophosphamide and corticosteroids	Progressive decrease in antibody titers; no transfusion since December 2000; hemoglobin level stable (11 g/dl)
10	3/00	16	Corticosteroids	No decrease in transfusion requirements
11	10/00	8	Corticosteroids	Slight decrease in antibody titers; no decrease in transfusion requirements
12	11/00	7	Immune globulin and corticosteroids	No decrease in transfusion requirements
13	8/00	10	No treatment	No decrease in transfusion requirements

\*Corticosteroids were usually given at a dose of 1 mg per kilogram of body weight per day and gradually reduced when a decrease in antierythropoietin antibodies was seen. Immune globulin (1 g per kilogram per day) was injected intravenously on two consecutive days. Cyclosporine was given at a dose of 100 mg twice a day. Cyclophosphamide was given as an intravenous bolus every four weeks for a total of three times.

ies is possible, since they are not monoclonal. The apparent dissociation affinity constant was 110 pM, and the maximal binding capacity was 0.6  $\mu$ g (60 U) of erythropoietin per milliliter (Fig. 3). This result was in good agreement with our rough estimates obtained from Figure 2. The same experiments were conducted with serum from all 13 patients, and antibodies with similar characteristics were found (Table 3). Two classes of binding sites were detected in the serum of the one patient (Patient 3) who had received epoetin beta exclusively.

Because erythropoietin molecules are heavily glycosylated and the glycosylation of recombinant molecules differs slightly from that of the endogenous molecule,<sup>5</sup> we tested the ability of antibodies to recognize the carbohydrate and the protein moieties of the molecule. Intact and deglycosylated <sup>125</sup>I-labeled

epoetin was incubated with patients' serum, and radioactivity bound to IgG was counted and analyzed by polyacrylamide-gel electrophoresis. Antibodies from all patients bound both glycosylated and deglycosylated <sup>125</sup>I-labeled epoetin with the same efficiency, showing that the antibodies were directed against the protein moiety of the erythropoietin molecule rather than the carbohydrate moiety (data not shown).

Serum from 12 of the 13 patients did not bind denatured <sup>125</sup>I-labeled epoetin, suggesting that the antibodies recognized conformational epitopes only. Serum from the patient treated only with epoetin beta (Patient 3) reproducibly bound denatured and deglycosylated <sup>125</sup>I-labeled epoetin. To evaluate this serum sample we used Scatchard analysis and either deglycosylated or deglycosylated denatured <sup>125</sup>I-labeled epoetin. Two binding sites with dissociation affinity

**TABLE 3.** CHARACTERISTICS OF ANTIERYTHROPOIETIN ANTIBODIES.

PATIENT No.	INHIBITION OF ERYTHROID-COLONY FORMATION*	REVERSAL OF INHIBITION†	BINDING CAPACITY‡	APPARENT DISSOCIATION AFFINITY CONSTANT§
			U/ml	pM
1	+	+	42	110
2	+	+	29	92
3	+	±	67	105, 410¶
4	+	±	86	350
5	+	+	36	100
6	±	ND	4	200
7	+	+	6	110
8	+	+	14	390
9	+	+	38	370
10	±	±	3	180
11	±	+	4	90
12	+	+	11	120
13	+	±	4	115

\*Inhibition of erythroid-colony formation was tested with use of normal bone marrow and 1 U of epoetin per milliliter. Plus signs indicate complete inhibition, with no colonies formed, and plus-minus signs partial inhibition, with fewer than 50 percent of colonies in cultures containing control serum formed in the presence of serum from the patients.

†Reversal of inhibition was assessed by adding 100 U of epoetin per milliliter to the culture. Plus signs indicate complete reversal, with the number of hemoglobinized colonies equal to that in control cultures, and plus-minus signs partial reversal, with some erythroid colonies formed, but not as many as in control cultures. ND denotes not done.

‡Binding capacity is expressed as the amount of radiolabeled epoetin bound per milliliter of the patient's serum after adjustment for the level of background activity. Binding was undetectable in control serum (binding capacity less than 200 mU per milliliter).

§The apparent dissociation affinity constant was determined with use of Scatchard analysis.

¶Patient 3 had two populations of antibodies with different binding affinities.

constants of 80 and 360 pM were found with deglycosylated <sup>125</sup>I-labeled epoetin. With deglycosylated denatured epoetin, a single class of binding sites with a dissociation affinity constant of 70 pM was observed, suggesting that this patient produced antibodies against both a linear and a conformational epitope of erythropoietin (data not shown).

**DISCUSSION**

We investigated the development of pure red-cell aplasia in 13 patients who were receiving epoetin as treatment for the anemia of chronic renal failure. All patients had received a diagnosis of pure red-cell aplasia within the preceding three years. After an initial response to epoetin, they became severely anemic and dependent on transfusions. In all patients, neutralizing antibodies against the protein moiety of epoetin were detected.

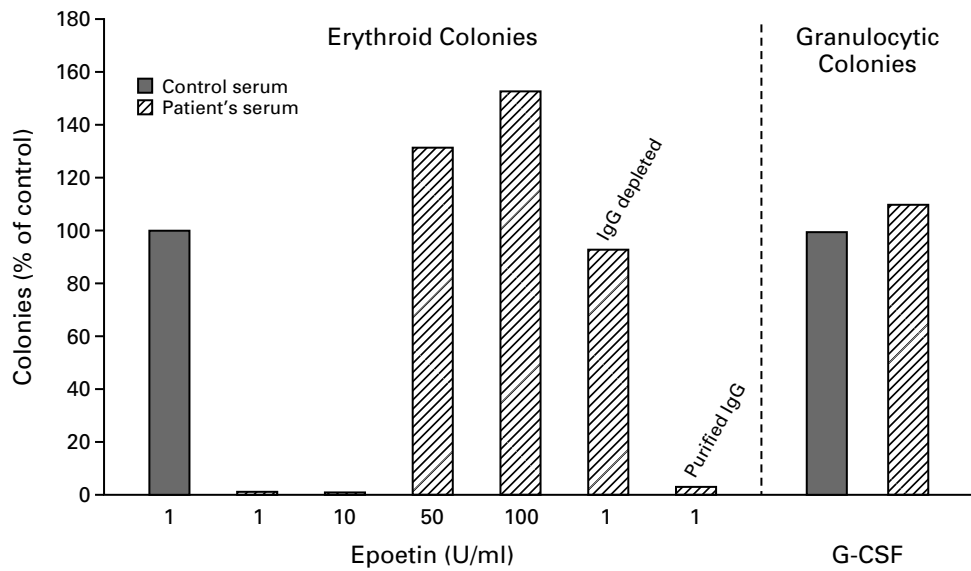
We have reported the presence of neutralizing antierythropoietin antibodies in a patient with pure red-cell aplasia who had never received epoetin.<sup>10</sup> We are not aware of similar cases and believe that neutralizing antibodies against erythropoietin are very rare. In the cases described here, the most plausible explanation is that the antierythropoietin antibodies were induced by epoetin therapy. This view is supported by the results of tests on serum samples from 4 of the 13 patients that were obtained from 1 to 15 months before the onset of pure red-cell aplasia. These samples did not contain detectable antierythropoietin activity.

We are aware of only three previous reports of antierythropoietin antibodies in patients receiving epoetin.<sup>7-9</sup> The identification within a three-year period of 13 patients in whom neutralizing antibodies against erythropoietin and pure red-cell aplasia developed during treatment with epoetin strongly suggests that the recombinant hormone had a role in causing the disorder. We do not, however, have information on how the hormone might trigger the formation of antierythropoietin antibodies. Of the 13 patients in our study, 11 were receiving epoetin alfa at the time of onset of anemia. Another patient had been receiving epoetin alfa and was switched to epoetin beta one month before the diagnosis of anemia. One patient received epoetin beta exclusively.

After epoetin therapy was stopped, there was a slow decline in antibody titers in all patients. Immunosuppressive treatment appeared to hasten the disappearance of the antibodies and might have allowed erythropoiesis to recover to the levels present before the initiation of epoetin treatment.

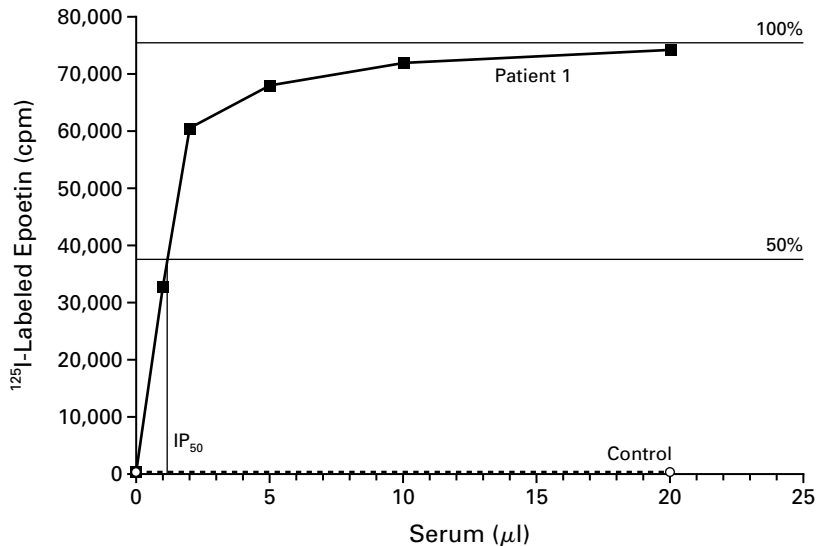
Scatchard analysis showed a linear pattern of erythropoietin binding by the antierythropoietin antibodies in each patient's serum. This result could have been due to the presence of a homogeneous population of antibodies, the recognition of a single epitope in erythropoietin by antierythropoietin antibodies, or both. The affinity of the antibodies for erythropoietin was slightly increased when they were tested with the use of deglycosylated epoetin, possibly reflecting some masking of epitopes by the bulky carbohydrate chains. Except for one patient who had been treated exclusively with epoetin beta, all patients had antibodies that recognized only conformational epitopes in the erythropoietin molecule — that is, no binding was noted after the denaturation of epoetin. Serum from the patient who had received only epoetin beta contained high-affinity antibodies that bound both native and denatured epoetin, a result suggesting that these antibodies were directed against both linear and conformational epitopes.

The antibodies in these 13 patients were able to neutralize very high concentrations of epoetin, and



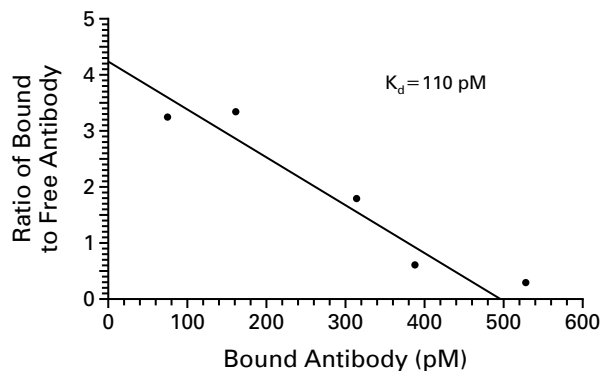
**Figure 1.** Inhibition of Erythroid-Colony Formation by Serum from Patient 1.

Erythroid cells in normal bone marrow were stimulated with 1 U of epoetin per milliliter in the presence of 20 percent pooled control serum; with 1, 10, 50, or 100 U of epoetin per milliliter in the presence of 20 percent serum from Patient 1; or with 1 U of epoetin per milliliter in the presence of 20 percent serum from Patient 1 in which IgG had been depleted with protein G Sepharose or in the presence of 100  $\mu$ g of purified IgG per milliliter from Patient 1. Erythroid colonies were scored after seven days of culture. The results are expressed as percentages of colonies formed in the presence of control serum and 1 U of epoetin per milliliter. Granulocytic colonies were grown for 14 days in 0.8 percent methylcellulose in Iscove's medium (Terry Fox Laboratories, Vancouver, B.C., Canada), containing 1 percent deionized bovine serum albumin and 200 ng of recombinant granulocyte colony-stimulating factor (G-CSF) (Amgen, Thousand Oaks, Calif.) per milliliter.



**Figure 2.** Binding of <sup>125</sup>I-Labeled Epoetin by Serum from Patient 1.

Binding capacity was calculated as the amount of serum from Patient 1 required to bind 50 percent of the radiolabeled epoetin after adjustment for the background level (IP<sub>50</sub>). Pooled serum from 10 healthy volunteers was used as a control.



**Figure 3.** Scatchard Analysis of Binding of <sup>125</sup>I-Labeled Epoetin to Patient's Antibodies.

Three microliters of serum from Patient 1 was incubated with various concentrations of <sup>125</sup>I-labeled epoetin in a total volume of 200  $\mu$ l of TRIS-buffered saline-Tween bovine serum albumin, and the degree of binding of antibody by the radiolabeled epoetin was determined after adjustment for the degree of nonspecific binding. Nonspecific binding of <sup>125</sup>I-labeled epoetin was determined with the use of 3  $\mu$ l of control serum instead of the patient's serum. The results were analyzed according to the method of Scatchard.<sup>14</sup>  $K_d$  denotes the dissociation affinity constant.

their affinity for erythropoietin is roughly similar to that of the erythropoietin receptor.<sup>15</sup> The efficient neutralization of erythropoietin by these antibodies most likely accounts for their ability to inhibit erythropoiesis in vitro and in vivo.

We recommend that patients receiving epoetin be tested for the presence of neutralizing antierythropoietin antibodies as soon as possible after the onset of unexplained anemia. If such antibodies are found, epoetin should be discontinued immediately. We do not recommend challenging these patients with another erythropoietic protein, since the antibodies that we found cross-reacted with all commercially available recombinant erythropoietic products (epoetin alfa, epoetin beta, and darbepoetin alfa) (Swanson S, Amgen; personal communication).

Although we did not detect such neutralizing antibodies in patients treated with epoetin for reasons other than the anemia of chronic renal failure, we cannot exclude the possibility of their development in such patients or in athletes who illegally use epoetin to enhance their performance ("blood doping"). The severity and duration of the anemia in our patients, necessitating red-cell transfusions, argue against the use of epoetin for unlicensed indications.

*Note added in proof:* During the preparation of our article, we identified nine more patients treated with epoetin alfa who presented with pure red-cell aplasia and similar neutralizing antierythropoietin antibodies.

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## APPENDIX

The other members of the study group were as follows: F. Kuentz and D. Lataillade (Association Grenobloise pour la Dialyse des Urémiques Chroniques, La Tronche, France); L. Mandart (Centre Hospitalier Prosper Chubert, Vannes, France); N. Dodd and P.E. Williams (Ipswich Hospital, Ipswich, United Kingdom); D. Durault and D. Besnier (Centre Hospitalier de St. Nazaire, St. Nazaire, France); B. Branger (Centre Hospitalier Universitaire Nîmes, Nîmes, France); V. Ribrag (Institut Gustave Roussy, Villejuif, France); A. Dürbach (Hôpital du Kremlin-Bicêtre, Le Kremlin-Bicêtre, France); L. Sutton and L. Mercadel (Hôpital La Pitié-Salpêtrière, Paris); and M.D. Pauti, C. d'Auzac, and S. Boudjeltia (Hôpital Européen Georges Pompidou, Paris).

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