Pure Red Cell Aplasia Induced by Erythropoiesis-Stimulating Agents

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Pure red cell aplasia in patients who are treated for anemia of chronic kidney disease with erythropoiesis-stimulating agents such as epoetin was first reported in 1998. Although the incidence of pure red cell aplasia peaked in 2002, it remains important for nephrologists to know how to investigate a suspected case of pure red cell aplasia and how to identify other causes of hyporesponsiveness to erythropoiesis-stimulating agents, which account for the vast majority of such cases. The authors reviewed the current status of information in the literature and drew on their personal experiences with patients regarding the diagnosis and management of epoetin-induced pure red cell aplasia. The mechanism for development of epoetin-induced pure red cell aplasia remains unconfirmed. It generally occurs after the production of neutralizing anti-erythropoietin antibodies. Elucidation of a suspected pure red cell aplasia case requires a systematic approach, beginning with simple measurements such as blood cell counts, because most cases of erythropoiesis-stimulating agent hyporesponsiveness are attributable to other causes. If these criteria indicate that the patient’s response to erythropoiesis-stimulating agent therapy is very poor, then bone marrow examination and measurement of anti-erythropoietin antibodies is justified. If pure red cell aplasia is confirmed, then cessation of erythropoiesis-stimulating agent therapy and initiation of immunosuppressive therapy are recommended. Continued study of epoetin-induced pure red cell aplasia is needed to help nephrologists prevent or manage future cases and will have implications for the use of other protein-based therapeutic agents.

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The development of pure red cell aplasia (PRCA) associated with erythropoiesis-stimulating agent (ESA) therapy was first recognized in 2002 (1). Most of the initial cases, reported by Casadevall et al. (1), were in patients who were treated with epoetin α manufactured by Ortho-Biotech (http://www.orthobiotech.com) outside the United States (Eprex, Erypo [Ortho Biologics, LLC, Manati, Puerto Rico]), but cases have since been reported with all commercially available ESA (2–7), including Epogen (http://www.amgen.com) and Aranesp (http://www.amgen.com) (both manufactured by Amgen, Inc., Thousand Oaks, CA), Procrit (http://www.orthobiotech.com [Amgen Inc.-Ortho Biotech, Thousand Oaks, CA, and Longmont, CO]), and NeoRecormon (http://www.roche.com [Roche Pharmaceuticals, Mannheim, Germany]). Although this complication of ESA therapy is extremely rare, the precise mechanism(s) responsible for the break in immune tolerance remains a mystery (8). It is important for nephrologists to know how to investigate a suspected PRCA case and to be aware that the vast majority of cases of hyporesponsiveness to ESA will not be due to antibody-mediated PRCA; therefore, the common causes of this condition should be investigated first, unless clear features of PRCA are present.

Diagnosis of ESA-Induced PRCA
PRCA is a rare hematologic disorder characterized by progressive, severe, normocytic, normochromic anemia of sudden on-
set; reticulocytopenia; and a striking, almost complete absence of erythroid precursor cells in the bone marrow (Table 1) (9). The blood reticulocyte count usually is very low (<10,000/μL), and hemoglobin levels decrease at a rate of approximately 0.1 g/dL per day (1 g/L per d), corresponding to the red blood cell lifespan; thus, patients rapidly become transfusion dependent. All other lineages are present and seem morphologically normal on blood smears. This pattern distinguishes PRCA from aplastic anemia, which usually involves all three cell lineages.

The hallmark of PRCA is the absence of erythroblasts from an otherwise normal bone marrow (Figure 1). In classic cases, the bone marrow aspirate and/or trephine biopsy shows a virtual absence of red cell precursors (in many cases <5% erythroblasts), whereas the cellularity of the bone marrow is normal, with normal myeloid cells and megakaryocytes. Because iron use is largely abolished in PRCA as a result of the absence of marrow erythropoietic activity, serum ferritin increases to very high levels, as does the transferrin saturation. Thus, serum ferritin levels of >1000 μg/L and transferrin saturation levels of >70% are characteristic of this condition.

PRCA is a primary hematologic disorder, but it can also occur secondary to various infections (especially human parvovirus B19), hematologic malignancies, chronic hemolytic anemias, autoimmune diseases (especially systemic lupus erythematosus, rheumatoid arthritis, and autoimmune hepatitis), thymoma, severe malnutrition, and exposure to a variety of drugs and toxins (10). Cases of PRCA have been reported in association with more than 30 drugs and chemicals, as summarized in Table 2, with most reports describing only one or two patients (11). A causal relationship has not been clearly proved in most cases. Causality may be established using the following three criteria (11): (1) at least five patients reported, (2) reports from at least three separate investigators, and (3) a minimum of one case of probable or higher causality using a published assessment scale (12). With this definition, there is sufficient evidence of causality for PRCA related to use of phenytoin, azathioprine, chlorpropamide, isoniazid, and ESA.

The pathogenesis of PRCA in most drug-induced cases is largely unknown, although the mechanism is believed to be related to direct effects on red cell precursors or induction of autoimmunity. In the case of ESA-induced PRCA, the pathogenetic mechanism has been shown clearly to be secondary to the development of neutralizing anti-erythropoietin antibodies (1). These antibodies, which recognize all available ESA (epoetin α, epoetin β, and darbepoetin α) as well as endogenous erythropoietin, block the interaction between erythropoietin and its receptor. Thus, identification of neutralizing antibodies is a key step in the diagnosis of ESA-induced PRCA. Conversely, in the Netherlands Cooperative Study on the Adequacy of Dialysis-2 (NECOSAD-2), 57 of 1677 patients who received hemodialysis were found to have inadequate epoetin response. The sera specimens of two of these 57 patients tested positive for anti-epoetin antibodies, but only one patient had clinical PRCA (13). These data indicate that the detection of anti-epoetin antibodies in circulation is not automatically associated with PRCA. In addition, Casadevall et al. (14) reported some cases in which classic bone marrow features of PRCA were not present, and indeed appreciable numbers of erythroid cells were seen, despite the presence of severe anemia and reticulocytopenia. In these cases, identification of anti-erythropoietin antibodies was essential to the diagnosis.

**Table 1. Features of ESA-associated antibody-mediated PRCA**

<table>
<thead>
<tr>
<th>Feature</th>
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<tr>
<td>Severe ESA resistance</td>
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<td>Transfusion dependence</td>
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<tr>
<td>Very low reticulocyte count (usually &lt;10 × 10⁹/L)</td>
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<tr>
<td>Usually normal white cell and platelet counts</td>
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<td>High serum ferritin and transferrin saturation levels</td>
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<tr>
<td>Bone marrow showing virtual absence of red cell precursors (&lt;5% of erythroblasts) in the presence of normal white cell and platelet maturation</td>
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<tr>
<td>Develops at least 2 mo after commencing ESA therapy (median approximately 10 mo)</td>
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<tr>
<td>Presence of antibodies directed against erythropoietin</td>
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*ESA, erythropoiesis-stimulating agent; PRCA, pure red cell aplasia.*

**Figure 1.** Hematologic stains of bone marrow (BM) from a normal patient (left) and a patient with antibody-mediated pure red cell aplasia (PRCA; right).

**Identification of Anti-Erythropoietin Antibodies**

Antibodies that are induced by therapeutic proteins, such as the ESA, may affect the safety, efficacy, and pharmacokinetic and pharmacodynamic characteristics of the proteins. It is therefore essential that procedures be identified or developed for detecting such antibodies and characterizing their important properties. Usually, some type of immunoassay is first used to screen samples (serum or plasma) for the presence of antibodies, and then positive screening results are confirmed, preferably by using an assay based on an alternative technology. Confirmed
positive samples are assessed for neutralizing characteristics using a bioassay.

A variety of assays (15) (Figure 2) have been used to detect anti-erythropoietin antibodies, including radioimmunoprecipitation (RIP) assays, ELISA, and surface plasmon resonance procedures (1,15–17). Each of these assays can yield informative results and has specific advantages and limitations. For example, ELISA (16) usually permit high throughput and are rapid, relatively easy to use, and relatively inexpensive; thus, they often are used as screening assays for antibody detection. However, they can produce nonspecific matrix effects, which usually manifest as false-positive results. Immobilization of the antigen may alter the conformation of the native protein, and ELISA may fail to detect “low-affinity” antibodies; both of these problems can lead to false-negative results. RIP assays measure antigen–antibody binding in the solution phase, which may be advantageous, and they can be highly sensitive and specific, but they are usually low-throughput assays that are difficult to automate. They require a source of radiolabeled antigen, which has a relatively short shelf life. RIP assays can be antibody isotype specific and may fail to detect rapidly dissociating antibodies. Surface plasmon resonance procedures for measuring anti-erythropoietin antibodies have been limited to Biacore (GE Healthcare Life-Sciences Corp., Amersham, UK) analysis methods (18). Such methods can be automated and specific but may be less sensitive than other binding assays. They can provide information on antibody kinetics, relative binding affinity, concentration, and isotype. For anti-erythropoietin antibodies, Biacore analysis enables detection of rapidly dissociating, low-affinity antibodies that are not detected by ELISA, RIP assays, and other non–real-time procedures; however, they require expensive, dedicated equipment. Also, immobilization of the antigen may alter the conformation of the native protein, and the regeneration step may degrade the antigen.

Bioassays are functional assays (Figure 3) that distinguish antibodies with neutralizing potential from those that simply bind to a protein such as erythropoietin but do not directly affect its biologic activity. Bioassay data may correlate with clinical response, whereas in vitro binding assay results may not. This distinction is important as a general concept but is particularly significant for erythropoietin and PRCA because

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### Table 2. Drugs and chemicals associated with PRCA

<table>
<thead>
<tr>
<th>Allopurinol</th>
<th>Aminopyrine</th>
<th>Arsenic</th>
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<tbody>
<tr>
<td>Azathioprine</td>
<td>Benzene hexachloride</td>
<td>Bromsulphalein</td>
</tr>
<tr>
<td>Calomel</td>
<td>Carbamazepine</td>
<td>Cephalothin</td>
</tr>
<tr>
<td>Chenopodium</td>
<td>Chloramphenicol</td>
<td>Chlorpropamide</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>Co-trimoxazole</td>
<td>Diphenylhydantoin</td>
</tr>
<tr>
<td>Erythropoietins</td>
<td>Estrogens</td>
<td>Fenofren</td>
</tr>
<tr>
<td>Gold</td>
<td>Halothane</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Leuprolide acetate and</td>
<td>Maloprim</td>
<td>Mepacrine</td>
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<tr>
<td>Chlormadinone acetate</td>
<td>(Dapsone and Pyrimethamine)</td>
<td>Pentachlorophenol</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>Mycophenolate mofetil</td>
<td>Phenobarbital</td>
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<tr>
<td>Penicillin</td>
<td>D-Penicillamine</td>
<td>Salicylazosulphapyridine</td>
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<tr>
<td>Phenylbutazone</td>
<td>Procainamide</td>
<td>Sodium valproate</td>
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<tr>
<td>Santonin</td>
<td>Sodium dipropyl acetate</td>
<td>Sodium valproate</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Sulfathiazole</td>
<td>Thiamphenicol</td>
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<td>Tolbutamide</td>
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**Figure 2.** The three antibody tests that have been developed for testing sera for the presence of anti-erythropoietin (anti-EPO) antibodies. (A) Radioimmunoprecipitation assay. (B) Biosensor immunoassay. (C) ELISA. Reprinted from reference (33), with permission.
patients who have developed ESA-induced, antibody-dependent PRCA have been found in the majority of the cases to produce erythropoietin-neutralizing antibodies. Bioassays are usually relatively time-consuming (because of long incubation times) and are prone to nonspecific matrix effects and interference by inhibitory molecules. Their sensitivity is moderate, and they can be relatively difficult to validate.

Erythropoietin bioassays that are used to measure neutralizing antibodies can be based on primary cultures of bone marrow–derived erythroid cells or on continuously growing, factor-dependent cell lines that proliferate in response to erythropoietin. Neutralizing antibodies present in serum from patients with ESA-induced PRCA block this erythropoietin-dependent cellular proliferation. Cell lines offer some advantages over primary bone marrow cells for erythropoietin bioassay. These include greater reproducibility and consistency of cell growth characteristics and erythropoietin responsiveness. Bone marrow samples may be available only in limited quantities, and the erythroid cultures need 7 d of incubation. Cell lines that have been used to demonstrate epoetin-neutralizing activity are TF-1 and U7-7, derived from patients with erythroleukemia, and 32D-EPO, a murine hematopoietic cell line transfected with the human erythropoietin receptor (19,20). Different erythropoietin-sensitive cell lines have different inherent sensitivities to the neutralizing effects of anti-erythropoietin antibodies in the bioassay; however, cell lines are sensitive to growth factors other than erythropoietin, and tight controls must be included in the bioassays.

It is important to elaborate a clear, prospective strategy for detecting and measuring erythropoietin antibodies and correlating results with clinical data relevant for diagnosis of PRCA. Purpose-specific validation, qualification, and standardization of assays for measuring and characterizing anti-erythropoietin antibodies are essential if meaningful results are to be produced.

In general, immunoassays for the detection of antibodies have target sensitivity of at least 500 ng/ml (so that if a patient has at least 500 ng/ml of anti-drug antibody in his or her circulation, then the assay would detect the patient as “positive”). Bioassays, because of their cell-based nature and higher inherent variability, have a target sensitivity of 1 μg/ml for neutralizing antibodies. It is important to note that assay sensitivity is based on the performance of a high-affinity positive control antibody and as such may not mimic the type of immune response generated in any given patient.

Suggested Clinical Approach to a Suspected PRCA Case
The first question that the nephrologist should ask is whether the patient’s poor response to ESA therapy is severe. To date, no cases of partial resistance to erythropoietin as a result of anti-erythropoietin antibodies have been reported. Thus, the clinical manifestation of PRCA generally behaves as an “all or none” phenomenon, and unless the patient has developed a fairly precipitous fall in hemoglobin, with a hemoglobin level of 5 to 6 g/dl or less and a reticulocyte count of less than 10 × 10⁶/L, it is likely that the patient’s ESA resistance has another cause. If, however, the patient has severe anemia and/or transfusion dependence with a very low reticulocyte count, then a bone marrow examination and measurement of erythropoietin antibodies is justified. The bone marrow examination in a patient with ESA-induced PRCA will usually confirm the presence of severe erythroid hypoplasia, but to meet the diagnostic criteria, the patient also must demonstrate the presence of circulating anti-erythropoietin antibodies. The absence of antibodies makes ESA-induced PRCA unlikely, even if PRCA is demonstrated on the bone marrow examination.

It is important to remember that other causes of anemia, such as iron deficiency and myelodysplastic syndromes, are much more common than ESA-induced PRCA in patients with chronic kidney disease (CKD) (21). The most common cause of ESA hyporesponsiveness is absolute or functional iron deficiency (Table 3). It is interesting that CKD has been associated with enhanced hepatocyte synthesis of hepcidin, which promotes functional iron deficiency by inhibiting the release of iron from reticuloendothelial cells to erythroid progenitors. ESA resistance is present in approximately 5 to 10% of patients, although interesting geographic differences exist across Europe and the United States. ESA resistance is evidenced by the persistence of anemia despite adequate dosing or by the requirement for high ESA dosages to achieve recommended he-

![Figure 3. An in vitro bioassay. Reprinted from reference (25), with permission.](image-url)

**Table 3. Main conditions associated with resistance to treatment with ESA**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tr>
<td>Iron deficiency</td>
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<tr>
<td>Chronic blood loss</td>
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<tr>
<td>Hyperparathyroidism/osteitis fibrosa</td>
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<tr>
<td>Aluminium toxicity</td>
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<tr>
<td>Hemoglobinopathies (e.g., α and β thalassemias, sickle cell anemia)</td>
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<tr>
<td>Vitamin deficiencies (e.g., folate or vitamin B₁₂ deficiency)</td>
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<tr>
<td>Multiple myeloma, myelofibrosis</td>
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<tr>
<td>Other malignancy</td>
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<tr>
<td>Malnutrition</td>
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<tr>
<td>Hemolysis</td>
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<tr>
<td>Inadequate dialysis</td>
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<tr>
<td>Adverse effects of certain drugs (e.g., cytotoxic and immunosuppressive agents, ACE inhibitors)</td>
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<tr>
<td>ESA-induced PRCA</td>
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ACE, angiotensin-converting enzyme.
moglobin targets (22,23). Clinical correlates of ESA resistance include female gender, malnutrition, presence of a tunneled catheter, overt vascular disease, and concomitant illness, but inflammation is recognized with increasing frequency as a cause of ESA hyporesponsiveness (24). The causes of inflammation in uremia are poorly understood but are likely to include multiple factors that are inherent in renal disease, including the presence of inflammatory cytokines. The inflammatory cytokines are believed to inhibit erythropoiesis directly, decrease erythropoietin production, and promote apoptosis of erythroid precursors.

**Treatment of ESA-Induced PRCA**

Almost all patients with PRCA require regular packed red cell transfusions until the PRCA resolves and the anti-erythropoietin antibodies disappear. Early recognition of PRCA and withholding of ESA therapy are essential, but cessation of ESA treatment alone generally will not cure ESA-induced PRCA (25). In cases of PRCA induced by other drugs (unrelated to anti-erythropoietin antibody production), the PRCA generally remits within 1 to 2 wk after elimination of exposure to the causative agent.

Patients who develop antibody-mediated PRCA in response to ESA treatment are unlikely to respond to treatment with other erythropoietic agents, because there is substantial cross-reactivity among erythropoietic agents, including endogenous erythropoietin (26). An exception to this rule was seen in a patient who had Eprex-associated PRCA and responded to darbepoetin α, without any complications, in the presence of persisting epoetin antibodies (3).

In patients with CKD and ESA-induced PRCA, immunosuppressive treatment is usually required to induce disappearance of anti-erythropoietin antibodies. Bennett et al. (27) reported recovery rates from PRCA of 2% without immunosuppressive therapy, 52% after immunosuppressive treatment(s) outside the renal transplantation setting, and 95% after kidney transplantation. The relative importance of immunosuppression and normalization of kidney function after kidney transplantation in resolving ESA-induced PRCA has not been established.

In the absence of kidney transplantation, treatment of PRCA includes oral administration of prednisone, usually at a starting dosage of 1 mg/kg per d. In patients with idiopathic PRCA, corticosteroids produce responses in approximately 50% of patients (28). Similar response rates were reported in patients with CKD and epoetin antibody–mediated PRCA. Verhelst et al. (29) reported higher recovery rates when corticosteroids were given together with cyclophosphamide (87%) than when corticosteroids were given alone. In patients with CKD and ESA-induced PRCA, oral cyclosporine therapy resulted in hematologic recovery in more than two thirds of the investigated PRCA cases (25). Of 15 patients with idiopathic PRCA treated with a humanized mAb to the IL-2 receptor (daclizumab), six (40%) responded to the treatment, but this therapy has not been evaluated in patients with antibody-mediated PRCA (30). Successful treatment with rituximab has also been reported in a few patients, whereas others failed to respond to this therapy (29,31). PRCA associated with parvovirus infection responds well to intravenous Ig therapy, but the results in patients with ESA-induced PRCA are poor (29).

**Reasons for the Increase in Cases of Eprex-Induced PRCA from 1998 through 2002**

Whereas only a few cases of ESA-induced PRCA were reported before 1998, between 1998 and 2004, almost 200 cases were described in patients who had CKD and were treated with ESA injected subcutaneously (5). Although cases have now been described with all formulations, the great majority occurred in patients who received epoetin (Eprex/Erypo) produced by Ortho Biotech and marketed outside the United States. The incidence rate rose drastically between 1998 and 2002, after a change in the formulation in which human serum albumin was eliminated and replaced by polysorbate-80 to avoid the risk for virus or previous transmission. Cases have been seen as early as 2 mo after therapy, with a median exposure time of approximately 9 mo.

The exact mechanism that leads to the development of antibody-induced PRCA remains unknown (32). Several hypotheses have been proposed to explain the disruption of B cell tolerance for an endogenous protein, but none has been confirmed (2,8,32). An acceptable explanation should be based on experimental data and a biologic rationale and should be in accordance with the epidemiologic data, such as the rarity of PRCA and its uneven geographic distribution. It was suggested that factors that could affect the protein during storage and handling might be responsible for the differences in the annual incidence of ESA-induced PRCA between countries (33).

The presence of epoetin-loaded micelles in the stored formulation has been suggested as a possible explanation (34). Although theoretically these micelles could present epoetin in an array form to B cells and thus enable breakage of tolerance, the micelles would be present in every syringe. Moreover, the micelles are unstable and are unlikely to be present long enough after injection to cause PRCA.

The concept that aggregates trigger antibody production is supported by clinical and experimental data (8,35–37). Periodicity of self-antigens, as is present in aggregates of proteins, seems to be very efficient in activating naïve or anergic B cells, which are responsible for tolerance.

It has been proposed that leachates released from rubber stoppers used only in syringes from Ortho Biotech could act as adjuvants and induce an immune response against erythropoetin, but experimental data substantiating this claim are controversial (27,33). The claim is based on animal experiments showing that concentrations of leachates that are much higher than what are present in the syringes increase the antibody response to ovalbumin in mice; however ovalbumin is a foreign protein in mice and the antibody response therefore based on a classical vaccine response, which disqualifies it as a model for breaking tolerance. The leachates are present in every syringe, which makes it difficult to explain both the rarity of PRCA considering the lack of genetic restriction in adjuvant responses and the geographic differences in incidence.
Consequences of ESA-Induced PRCA for the Use of Biopharmaceuticals

As stated, whereas the peak of Eprex-induced PRCA cases occurred >4 yr ago, the debate on what triggered the autoimmune disorder continues today. The association with a change in formulation makes PRCA of interest to the biotechnology industry as well as the medical community because it raises the broader question of the potential immunogenicity of biopharmaceuticals in general. The medical use of proteins has a long history. It started more than a century ago, when immune sera of animal origin were introduced for the prevention or treatment of infections, followed some decades later by the use of insulin of porcine and bovine origin. These products were immunogenic in humans, sometimes even leading to serious anaphylactic reactions. These adverse effects were easily explained by the foreign nature of the proteins, leading to a classical immune reaction. With the development of recombinant DNA technology, the large-scale production of homologs of human protein products, such as the interferons, growth factors, and hormones, became feasible, resulting in their application in a large number of patients. It came as a surprise that these products, to which patients are immune tolerant to the endogenous forms, also induced antibodies.

The mechanisms by which tolerance is induced or broken are not completely understood. Many factors have been reported to influence immunogenicity, but the presence of aggregates as triggers for B cell response is supported by clinical and experimental data. In a few cases, it has been shown that periodicity of self-antigens present in aggregates is efficient in activating naïve or anergic B cells, which are responsible for tolerance (38).

Conclusions

The elucidation of a suspected PRCA case is usually not difficult, but it requires a systematic approach to its investigation. Simple measurements such as the hemoglobin level and counts of reticulocytes, white cells, and platelets may be helpful as a starting point. More detailed investigations such as bone marrow examination and erythropoietin antibody measurement should be reserved for cases in which there is a reasonable suspicion of antibody-mediated PRCA. Once a patient has received a diagnosis of ESA-induced PRCA, treatment includes cessation of ESA therapy and initiation of immunosuppressive therapy. Rechallenge with a different epoetin preparation should be considered with caution and only when anti-epoetin antibody levels have become undetectable.

Disclosures

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References

14. Casadevall N, Rossert J, Swanson S: Anti-erythropoietin (EPO) antibodies (Abs) not associated with pure red cell aplasia (PRCA), in patients treated with erythropoiesis-stimulating agents (ESAs). Poster presented at the annual meeting of the American Society of Nephrology; October 29 through November 1, 2004; St. Louis, MO
16. Hoesel W, Gross J, Moller R: Development and evaluation of a new ELISA for the detection and quantification of