What is antibody-mediated pure red cell aplasia (PRCA)?

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Abstract
Antibody (Ab)-mediated pure red cell aplasia (PRCA) is an immunological pathology associated with the production of neutralizing Abs that inhibit the erythropoietic activity of endogenous erythropoietin (EPO) and recombinant erythropoiesis-stimulating agents (ESAs). Although this disorder occurs very rarely, the number of reported cases has increased dramatically in recent years, predominantly in patients with chronic kidney disease (CKD)-associated anaemia receiving subcutaneous (s.c.) injections of one particular formulation of recombinant epoetin-α.

This disorder is differentiated from classic forms of PRCA that are caused by chemical toxaemia (i.e. erythroblastopenia induced by chemical compounds), lymphoproliferative neoplasms, thymoma, human parvovirus B19 and certain autoimmune disorders.

Patients with Ab-mediated PRCA develop resistance to EPO and severe anaemia that follows a period of successful erythropoietic response, and exhibit characteristic decreases in blood haemoglobin (Hb) level and in the number of circulating reticulocytes. However, it is not yet possible to predict which patients will develop PRCA or when in the course of their treatments PRCA may develop. Laboratory confirmation of Ab-mediated PRCA requires bone marrow examination demonstrating few or no erythroid precursors and the presence of serum anti-EPO Abs using a validated assay. These neutralizing anti-EPO Abs recognize the protein core of the EPO molecule; carbohydrate groups on EPO can affect the binding of Abs but are themselves not immunological determinants. Animal models are being developed to increase further our understanding of the immunological mechanisms underlying the onset and progression of Ab-mediated PRCA.

Keywords: aetiology of PRCA; erythropoietin; pure red cell aplasia

Introduction
Erythropoietin (EPO) is a sialoglycoprotein growth factor that stimulates and controls the maturational development and proliferation of erythroid progenitors in the adult bone marrow, leading to production of circulating red blood cells [1]. In addition, this protein has been found to be a key factor in the development of the embryonic central nervous system and cardiovasculature, and has pleiotropic effects in angiogenesis and neurocognition [2,3]. Erythropoietin gene expression and EPO synthesis in the kidney is stimulated by hypoxia under regulatory control of tissue hypoxia factor-1 (HIF-1) [4,5].

Recombinant EPO has been used successfully for >12 years for the treatment of anaemia associated with chronic kidney disease (CKD) or cancer therapy [6–8]. The structural and functional similarities of different recombinant EPOs to endogenous EPO [9] are the foundation for the clinical usefulness of these biopharmaceutical agents. Various recombinant EPOs (e.g. epoetin-α, epoetin-β, darbepoetin-α, etc.) can be distinguished from each other and from endogenous EPO based on microheterogeneity of the carbohydrate groups attached to the protein core of the molecule [10]. Darbepoetin-α is a modified epoetin with five N-carbohydrate chains instead of three present in epoetin-α and -β.

Attention has been focused recently on the development of pure red cell aplasia (PRCA) in some patients with CKD that received recombinant epoetin-α. This rare condition develops suddenly and is caused by the production of EPO-neutralizing antibodies (Abs) that eliminate the biological activity of the recombinant protein as well as of endogenous EPO. Patients with this disorder become characteristically EPO refractory and transfusion dependent [11].

This article describes the characteristic clinical symptoms and diagnostic features of Ab-mediated PRCA that distinguish it from classic PRCA and aplastic anaemia. Data are also presented on the binding of neutralizing Abs to epoetin and the development of experimental models of PRCA.
Haematological effects of EPO

The production of mature circulating red blood cells (RBCs) from erythroid progenitors during haematopoiesis in the bone marrow is a complex and tightly regulated process that proceeds through a cascade of cellular events and differential growth factor activation (Figure 1). Temporally controlled physiological combinations of stem cell factor (SCFγ), interleukin (IL)-3 and EPO stimulate the differentiation and proliferation of early erythroid progenitors (‘burst-forming units erythroid’ or BFU-E) to late-stage progenitors (‘colony-forming units erythroid’ or CFU-E) and mature RBCs (Figure 2) [4,12–14]. Consequently, increased EPO production results in expansion of erythroid cells in the bone marrow and increased numbers of circulating reticulocytes and RBCs, while abnormally low EPO concentrations result in anaemia. According to the model proposed recently by Khoury and Bondourant, physiological concentrations of EPO also promote the survival of late-stage CFU-E during their terminal differentiation into EPO-independent reticulocytes and mature RBCs by retarding cellular apoptosis (programmed cell death) [14].

Normal embryogenesis and erythropoiesis require expression of both functional EPO and EPO receptor (EpoR) genes [15,16]. The pleiotropic effects of EPO as an autocrine, paracrine and endocrine growth factor derive from the wide tissue distribution of the EpoR [3]. Mice that are homozygously deficient for either the EPO or EpoR genes possess normal levels of erythropoiesis, cellular EPO responsiveness, blood haemoglobin (Hb) levels and other haematological parameters under normal conditions and in the absence of haematological stress [16]. Functional EPO and EpoR genes are required for the survival and differentiation of committed CFU-E precursors [15].

Pure red cell aplasia

PRCA is a severe haematological disorder that is characterized by the absence of erythroid precursor cells in bone marrow samples, or maturational arrest of erythroid cells [19]. Although this disorder was...
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Described >80 years ago [19], a more complete description of the various aetiologies of PRCA was not available until the publication of a series of original papers and review articles by Krantz and colleagues beginning in the late 1960s [20–23]. Classic PRCA is either congenital (Diamond-Blackfan syndrome) or acquired. Acquired PRCA can be primary in nature [idiopathic (often due to an autoimmune mechanism) or pre-leukaemic] or secondary to thymoma, haematological malignancies, chemical- or drug-associated toxoaemia, B19 parvovirus infection or several other aetiologies such as autoimmune disorders [19]. Arrest of erythropoiesis leading to PRCA can be induced by cellular inhibition of the differentiation and survival of erythroid progenitor cells and erythroblasts or by soluble inhibitors (e.g. antibodies) directed against the erythroid progenitor and erythroblast or very rarely against the EPO molecule.

PRCA is a rare condition, with only ~600 cases reported in the literature up to 1990 [19]. Idiopathic PRCA was also an obscure disorder; early studies of patients with PRCA revealed the presence of Abs in plasma that specifically recognized erythroblast nuclei and inhibited haem synthesis [20,21]. PRCA due to auto-AbS against erythropoietin was very rarely reported and only four cases of auto-Ab-mediated PRCA were reported between 1975 and 1999 [24–27]. None of these patients had been treated with exogenous EPOs. The first use of recombinant epoetins began in 1998. During the first 10 years of therapeutic use of epoetins, only three cases of PRCA were described in patients that received recombinant EPO for CKD-associated anaemia [28–30].

This clinical picture changed suddenly in 1999 with the reported increase in cases of PRCA that were linked to neutralizing Ab responses to recombinant EPO in patients with CKD [31,32]. The first case series of patients (n = 13) with the Ab-mediated form of PRCA were reported in Europe by Casadevall and colleagues in 2002 [32].

Initially, the causative factors associated with this immunological form of PRCA were poorly understood. Patients had responded initially to the erythropoietic effects of exogenous therapeutic EPO for varying periods (months to years), as judged by its ability to stimulate production of normal blood Hb levels and reticulocyte counts. In these patients, response to exogenous EPO began to decline suddenly during the course of treatment, with no response that could be elicited by increased dosing of EPO or by being switched to another brand of EPO.

### Natural history of Ab-mediated PRCA

The natural history of Ab-mediated PRCA is variable in terms of the time to onset of anaemia and patients' course of treatment. For example, this disorder may not appear for months to years after beginning treatment with recombinant ESAs (9 months, average; range: 2–64) [33], thus making it difficult to predict when a patient may begin developing PRCA. One of the main confounding factors of Ab-mediated PRCA is the rapid onset of EPO-refractory anaemia in patients that previously had responded well to exogenous EPO treatment. In many cases, further treatment with equal or increased doses of epoetin or other ESAs is continued past the time anaemia develops, but without erythropoietic response. In terms of product factors and route of administration, one formulation of epoetin-α (Eprex®, Ortho Biotech LLC, Manati, Puerto Rico) administered subcutaneously (s.c.) has been found to be associated with the majority of cases [34,35].

Analysis of the serological and haematological profiles and treatment histories of this cohort of patients has demonstrated some characteristic symptoms of Ab-mediated PRCA (Table 1). The most clinically significant haematological consequences of PRCA include the rapid decline in blood Hb levels that often requires periodic transfusions (up to four units of packed RBCs per month) to maintain levels of circulating Hb, and the corresponding drop in circulating reticulocytes. These two parameters are important indicators of possible PRCA that should be confirmed haematologically. The decline in blood Hb level of ~0.1 g/dl per day in patients with PRCA allows for an estimate of when RBC production ceased.

Confirmation of Ab-mediated PRCA requires each of the following criteria to be met [11,33]: (i) most frequent causes of PRCA are excluded; (ii) bone marrow examination shows <5% erythroid precursors (Figure 3); and (iii) EPO-binding Abs confirmed with a validated and qualified assay, such as radioimmuno-precipitation (RIP) assay [36].

### Immunology

#### Ab specificity

All recombinant therapeutic proteins have the capacity to stimulate the production of Abs, but the incidence of Ab production and the types of Abs formed in patients vary widely among the different agents. Consequently, the biological effects of Ab production on these various agents vary considerably [37,38].

The first immunochemical characterizations of Abs against erythropoietin were done by Casadevall and colleagues in 2002 [32]. Serum from the 13 patients recognized the native molecule but also the completely deglycosylated molecule, and consequently the Abs

<table>
<thead>
<tr>
<th>Table 1. Characteristics of antibody-mediated PRCA</th>
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<tr>
<td>Anaemia with normal or elevated mean corpuscular volume</td>
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<tr>
<td>Reticulocytopenia (&lt;10 000/μl)</td>
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<td>Normal numbers of circulating platelets and white blood cells</td>
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<td>Less than 5% erythroblasts in the bone marrow, with otherwise normal cellularity</td>
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were directed against the protein core of the molecule [32]. Serum from 12 of the 13 patients reported in this study did not bind to detergent-denatured EPO, indicating the specificity of their Abs for a conformational epitope on EPO. Serum from a patient with epoetin-β-induced PRCA had both conformational and non-conformational anti-EPO Abs. These results support the observation that Abs against one ESA will bind to and inhibit the activity of another chemically related ESA. Therefore, as noted in several case histories [39,40] and in patients described in previous case series [41], switching to other ESAs does not reactivate erythropoiesis because of the cross-neutralizing capacity of the anti-EPO Abs.

Results of bioassays have shown that Abs in serum from patients with full-blown PRCA are high affinity in nature and can effectively neutralize high concentrations of EPO [32]. In the example provided by Casadevall and colleagues [32], high-affinity IgG Abs in 1 ml of serum from a patient with PRCA effectively neutralized up to 10 IU of exogenous EPO in a bioassay (Figure 4). Depletion of the IgG Abs in this serum effectively reversed the Ab-mediated inhibition of erythroid cells proliferation in vitro. In this assay, anti-EPO Abs had no effect on differentiation of granulocytic precursors.

However, the presence of anti-EPO Abs in serum is not absolutely predictive of PRCA: some epoetin-treated patients possess anti-EPO Abs without any symptoms or signs of Ab-mediated PRCA [42]. In addition, low-affinity Abs detected by a different technique (BIAcore) have been found in some baseline serum samples from patients without PRCA [43]. The immunological mechanisms responsible for developing Ab-mediated PRCA are not known.

**Treatment cessation following development of Ab-mediated PRCA**

In some patients with Ab-mediated PRCA, cessation of epoetin treatment alone is sufficient to allow...
erythropoietic recovery. However, in the majority of reported cases, some form of immunosuppressive therapy (e.g. corticosteroids) is required to repress Ab formation and stimulate erythropoiesis [41]. The protracted decline in anti-EPO Abs following cessation of epoetin treatment in a patient with Ab-mediated PRCA is shown in Figure 5. This figure shows that the patient’s circulating reticulocytes and serum EPO levels did not rebound until ~40 weeks post-cessation of treatment and after immunosuppressive therapy, and when the anti-EPO Abs (measured by RIP assay) had declined to a binding capacity of <1 μU of EPO/ml of serum. Thus, this patient did not recover from Ab-mediated PRCA until many months after EPO treatment was stopped and when anti-EPO Abs had declined to nearly undetectable levels.

**Conclusions**

PRCA was once described in the literature as an uncommon disorder. It is now recognized as a severe, refractory anaemia in rare patients receiving recombinant ESAs for CKD-associated anaemia. The clinical history and diagnostic criteria for Ab-mediated PRCA are now more clearly defined than before, with confirmation of the disorder requiring bone marrow examination and assay results demonstrating the presence of anti-EPO Abs in serum. A sudden decrease in blood Hb level and number of circulating reticulocytes indicate the possible onset of Ab-mediated PRCA. Antibodies against one recombinant product cross-neutralize other ESAs, which limits treatment options in these patients and precludes switching to other products. Without re-treatment options and the possibility of renal transplantation, patients with Ab-mediated PRCA inevitably become transfusion dependent.

Although the majority of cases of Ab-mediated PRCA have been associated with one formulation of epoetin-α outside the USA, the immunological mechanisms by which neutralizing Abs are formed against recombinant EPO are not understood. Experimental animal models of EPO-induced anaemia are being developed for the purpose of investigating the immunological and immunochemical determinants of Ab-mediated PRCA. It is therefore not yet possible to predict which patients may produce Abs against their erythropoietic agent or which correlates of protein immunogenicity help determine the tendency of patients to develop Ab-mediated PRCA.

**Conflict of interest statement.** The author has received honorarium and/or research grants from Amgen, Roche, and Ortho Biotech.

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