Granulocyte colony-stimulating factor (G-CSF) is a growth factor stimulating the proliferation and differentiation of hematopoietic progenitor cells committed to the granulocyte lineage. As a recombinant human protein (rhuG-CSF), it is widely used for the treatment of patients with neutropenia (e.g., after chemotherapy) with a low total leukocyte count (TLC). Moreover, rhuG-CSF is given to increase the number of circulating stem cells for marrow harvest. It is generally well tolerated and serious adverse events are rare. Nevertheless, complications have been described including cutaneous vasculitis and neutrophilic dermatosis. In addition, in patients with autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, disease flares have been described when using rhuG-CSF for the treatment of neutropenia or for stem cell collection. So far, no complications have been reported in patients with antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis (AASV) and rhuG-CSF administration. AASV are diseases characterized by numerous autoimmune phenomena, probably in response to infectious agents as the initiating event. In particular the activation of polymorphonuclear neutrophils (PMN) might play a major role in the pathogenesis: expression of MHC class II antigens CD80 and CD86, receptors normally found only on antigen-presenting cells (dendritic cells or monocytes), have been described on circulating PMN of patients with clinically active vasculitis, as well as an upregulation of activation-associated surface receptors such as CD64, CD63, and CD66b, or the integrin adhesion molecules CD11b/CD18. Moreover, a correlation was found between disease activity and surface proteinase-3 expression (PR3, one of the main targets of ANCA in patients with AASV) on PMN, as well as activated PMN in renal biopsies, and their numbers correlated with the extent of impaired renal function. Together, these findings suggest that activated PMN are crucial for initiating the disease.

The potential risk of tissue damage by inappropriately activated PMN is well recognized. During infections, activated PMN normally emigrate to the infected sites to release their proinflammatory cytokines or to generate cytotoxic mediators. By activation in the microvasculature, in contrast, they release their mediators in the immediate vicinity of endothelial cells and can thereby cause damage to the vascular bed, eventually resulting in vasculitis.
Moreover, due to de novo synthesis of cytokines and surface receptors the functional repertoire of PMN expands during the inflammation\textsuperscript{12}. Thus the participation of PMN in the pathogenesis of AASV is well established.

We describe 2 patients with AASV with disease flare during rhuG-CSF therapy.

\section*{CASE REPORTS}

\textbf{Case 1.} A 40-year-old woman was diagnosed with AASV in 1996 with initial manifestations including the eye, ear, and lung. In addition, PR3-ANCA was found. After conventional therapies including oral and intravenous cyclophosphamide (CYC), prednisone, azathioprine, trimethoprim/sulfamethoxazole, anti-tumor necrosis factor-\(\alpha\) (etanercept), and methotrexate, high-dose IV CYC was given to induce remission for a therapy-refractive pseudotumor behind the right eye. In parallel, stem cell collection was planned, for which the patient received 2 g/m\textsuperscript{2} IV CYC (absolute 4 g) and rhuG-CSF was administered subcutaneously (300 \(\mu\)g for cell mobilization. On Day 12, she developed a massive painful swelling of the right eye; C-reactive protein (CRP) increased to 22.4 mg/dl and the TLC to 35,400/\(\mu\)l. A computed tomography (CT) scan showed an intense increase of the retroorbital mass (Figure 1). A biopsy excluded bacterial abscess but an active vasculitis was diagnosed. In addition she complained about severe dyspnea and a stridor. A subglottic tracheal stenosis was found by laryngoscopy. A prednisone pulse of 100 mg/day was started, which led to regression of the orbital swelling and respiratory symptoms. Because of incomplete cell collection the procedure had to be repeated. Again, the patient received rhuG-CSF (without CYC) and once more a remarkable swelling of the right eye occurred, which again remitted after a prednisone pulse. The titer of PR3-ANCA measured by ELISA remained unchanged throughout.

\textbf{Case 2.} In 1999, AASV was diagnosed in a 52-year-old man with initial manifestations including sinusitis and pulmonary infiltration and positive PR3-ANCA. After a renal relapse in 2002, he was treated by CYC pulses and a stem cell collection was performed, for which he received 3 g IV CYC. Because we were aware of the disease flare experienced by Patient 1 after administration of rhuG-CSF, Patient 2 received a lesser dose: only 4 subcutaneous injections of 300 \(\mu\)g filgrastim, starting after he reached the leukocyte nadir at Day 10 (TLC 1500/\(\mu\)l). In addition, he received 50 mg/day prednisone as a precaution. Cell collection was performed successfully on Day 13 (recovery: 8.45 \(\times\) 10\textsuperscript{6} CD34+ cells/kg). At Day 26 (TLC 10,700/\(\mu\)l) he complained of cough and hemoptysis. A chest radiograph showed a new focal consolidation in the left lung, indicative of either pneumonia or manifestation of AASV. In parallel, CRP increased from 0.8 to 3.6 mg/dl and the PR3-ANCA titer increased from 12 to 22 U/ml (normal < 2 U/ml). After 2 different antibiotic therapy regimens (ampicillin/sulbactam and clarithromycin/ciprofloxacin) the consolidation was still detectable by a CT scan, and CRP was persistently elevated (1.9 mg/dl). In August 2002, his therapy was modified to more intensive immunosuppression (oral CYC 100 mg/day). A followup CT in October 2002 showed a total remission of the pulmonary infiltrate (Figure 2). Based on these findings, an infectious condition was ruled out and granuloma formation due to underlying disease was presumed.

\section*{DISCUSSION}

RhuG-CSF is commonly used for treatment of neutropenia as well as for the mobilization of progenitor cells for stem cell collection. Its efficacy and potential risks have been reviewed\textsuperscript{1}. Although rhuG-CSF is well tolerated in most patients, complications have been described, including necrotizing and leukocytoclastic vasculitis. These findings appeared to be related to the fact that rhuG-CSF as a hematopoietic growth factor raises the number of circulating PMN and affects various PMN functions. According to current concepts of the pathogenesis of AASV, PMN are central effector cells in initiating the disease, although the activation pathway is still a matter of speculation. In addition, because clinical flares have been described when using rhuG-CSF in patients with autoimmune diseases, caution has been advocated in patients with AASV. Nevertheless, Hellmich, et al have shown that treatment with rhuG-CSF was safe in patients with AASV and a CYC-induced neutropenia with no signs of disease reactivation during a 4-week followup. The administration was clinically beneficial, with fewer infections in the rhuG-CSF group\textsuperscript{11}.

We describe 2 patients with AASV developing a localized flare after administration of rhuG-CSF for stem cell mobilization. These cells were needed for autologous stem cell transplant (ASCT), a rescue treatment for severe autoimmune disease\textsuperscript{14}. Patients with vasculitides have also undergone ASCT in phase I and II trials, and complications have not been reported so far\textsuperscript{14,15}. We intended to collect

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{Patient 1: CT scan of the orbita before administration (A) and after disease flare after rhu-G-CSF administration (B). Note the disease progression, with infiltration of the orbital fat and eye muscle, encasement of the orbital nerve, and destruction of the medial orbital wall.}
\end{figure}
stem cells (without ASCT) for a rescue-backup before initiating CYC therapy in both patients, because longterm CYC treatment is associated with myelodysplastic syndrome.

How the relapses are related to the rhuG-CSF treatment is a matter of speculation. One possibility is that rhuG-CSF increases the absolute number of circulating PMN and thus their cytotoxic potential. But as well, neutrophilic functions are altered in many ways by G-CSF. Our observations (data not shown) and data from others show that PMN activation occurs after starting rhuG-CSF treatment in vivo: a significant increment of the surface receptors CD11b/CD18, CD14, CD16, CD64, and CD66b is seen, as well as an increase of elastase antigen levels in blood. Moreover, rhuG-CSF primes PMN for respiratory burst and stimulates phagocytosis and antibody-dependent cellular cytotoxicity. This might lead to an enhanced proinflammatory potential resulting in increased adherence to vascular endothelium and consequently in damage to the endothelium and/or vulnerable tissues. These in vivo observations suggest that the disease flares in our patients were most probably induced by an increase and activation of circulating PMN.

Hellmich, et al emphasize that complications of rhuG-CSF treatment mainly arise in patients after longterm administration, when the TLC increases over 800/µl. They postulate that duration of the rhuG-CSF treatment and TLC are important determinations for complications. Additionally they showed that G-CSF (in contrast to GM-CSF) did not induce PR3 membrane expression on PMN in vitro, which might explain why rhuG-CSF administration was safe in their population of patients with AASV.

Unlike Hellmich, et al, who used the rhuG-CSF treatment to increase the TLC just high enough to prevent infections, we hoped to enhance the yield of CD34+ precursor cells during stem cell mobilization, which is dependent on the absolute number of circulating cells. Standard protocols recommend early and intensive stimulation with high rhuG-CSF doses. In our patients with AASV, this procedure apparently induces flares. Even lower doses and concomitant therapy with prednisone for Patient 2 were not sufficient to prevent a flare, although the symptoms appeared less severe than in Patient 1.

In conclusion, when using rhuG-CSF in patients with AASV and neutropenia or for stem cell mobilization, the duration and dosage should be limited to a minimum and the potential for disease flares should be recognized. Our clinical observations emphasize the relevance of activated PMN in the pathogenesis of AASV.

REFERENCES


