Correspondence

PROLONGED COMPLETE REMISSION IN TWO CASES OF ACUTE PROMYELOCYTIC LEUKAEMIA TREATED WITH ATRA ALONE

The use of all-trans retinoic acid (ATRA) therapy for induction for acute promyelocytic leukaemia (APL) is well established and leads to a complete remission rate of > 70%. However, remissions are short and consolidation with chemotherapy is required. Continuous treatment with ATRA has been associated with a decrease in plasma concentrations of the drug, which may lead to resistance. Recently, a case has been reported successfully treated with ATRA alone, followed by maintenance with intermittent ATRA and continuous low-dose methotrexate and 6-mercaptopurine (Sanz et al., 2000). Here, we report two cases of APL with prolonged remissions after treatment with ATRA alone.

An 81-year-old woman presented with pancytopenia due to APL in October, 1992. Cytogenetic analysis revealed t(15;17) as the sole abnormality, and the rearrangement was confirmed by Southern blot analysis. She was started on ATRA at 45 mg/m². Chemotherapy was not given because of her age and history of cardiovascular disease. Her leucocyte count increased to 25 x 10⁹/l on day 1. On day 10, bilateral pleural effusions and culture negative fever developed, which was treated successfully with dexamethasone. The dose of ATRA was decreased to 10 mg/m² on day 30 because of abnormal liver function tests, and she remained on this dose for another 95 d. Bone marrow on day 73 showed morphological remission with persistence of t(15;17). No further bone marrow examinations were performed, but her blood counts remained normal, and reverse transcription-polymerase chain reaction (RT-PCR) for the PML/RARα transcript on peripheral blood was negative, with normal blood counts assessed 4 and 6 years after diagnosis. She died of unrelated cardiovascular complications in November 1999, 7 years after diagnosis.

The second case involved a 76-year-old woman who presented in September 1997 with extensive mucosal bleeding. Laboratory investigations showed pancytopenia and disseminated intravascular coagulation due to APL, and RT-PCR confirmed the presence of the short isoform of the hybrid transcript generated by t(15;17). She was treated with ATRA at 45 mg/m² for 149 d. She declined treatment with chemotherapy. Her coagulation parameters normalized by day 2, and the leucocyte count reached a peak of 40 x 10⁹/l on day 12. She developed a cough and dyspnoea, which responded to dexamethasone. By discharge on day 18, she was feeling well with normal blood counts except for platelets at 60 x 10⁹/l; by day 35, the platelet count was normal. On day 58, bone marrow showed morphological remission, with normal cytogenetics and negative RT-PCR. Further RT-PCR analyses on peripheral blood have remained negative, most recently performed in July 2001. The patient remains alive and well with normal blood counts as of September 2001, 4 years after diagnosis.

The mechanism by which our patients achieved prolonged complete remissions is unclear. It is unlikely that we observed spontaneous remissions as they are rare and of short duration (Enck, 1985). It is likely that ATRA was important in inducing the remissions.

ATRA in supraphysiological doses causes differentiation of the promyelocyte by dissociation of a transcriptional corepressor complex, that exerts a dominant-negative effect on normal RAR-α-regulated gene transcription (Melnick & Licht, 1999). Continuous treatment leads to decreased plasma concentration and clinical resistance. However, leukaemic cells from relapses can continue to differentiate in vitro, though not in vivo. Moreover, the efficacy of liposomal ATRA suggests that the intracellular concentration of ATRA is critical, as use of this formulation maintains plasma concentrations of tretinoin and response in relapsed patients, but the response does not correlate with plasma concentration (Estey et al., 1996). Furthermore, the use of liposomal ATRA alone can induce prolonged molecular remissions (Estey et al., 1999). Thus, maintaining adequate intracellular concentrations of retinoids in the relevant target cell may permit ongoing differentiation.

The prolonged complete responses in our patients indicate that the metabolism of ATRA in some patients may be endogenously altered to permit persistent and clinically effective intracellular concentrations of the ligand. Alternatively, the unusual response to ATRA may indicate that the disease pathogenesis in our patients may have differed from the norm, as it is clear from animal models that the translocation is necessary but not sufficient to produce leukaemia, and that other cytogenetically silent lesions must contribute (Zimonjic et al., 2000). The mechanisms mediating induced differentiation by ATRA have been intensively investigated (Melnick & Licht, 1999), but only recently has the means of cell death been elucidated (Altucci et al., 2001). It is conceivable that differing clinical outcomes to ATRA treatment may be due to intrinsic variations in leukaemic stem cells in their susceptibility to retinoid-induced, TRAIL/DR5-mediated paracrine apoptosis.
Although aplastic anaemia (AA) is thought to be mediated by an autoimmune destruction of haematopoiesis (Young, 2000), the aetiology of autoimmunity in AA is not fully understood. Some cases of acquired AA are an idiosyncratic consequence of exposure to certain drugs and chemicals but, even in this context, the mechanisms by which xenobiotics trigger the immune system have yet to be determined. Altered drug disposition and metabolism may be responsible for increased susceptibility, and pharmacogenetic variations are possible risk factors for idiosyncratic drug-induced AA (Marsh et al., 1999). The MDR1 gene encoded P-glycoprotein (P-gp), linked to multidrug resistance in leukaemia and expressed by normal haematopoietic cells, protects stem cells from toxins by actively extruding a variety of drugs (Chaudhary & Roninson, 1991). We observed previously that P-gp function was significantly reduced in T-cells (Calado et al., 1998) and bone marrow CD34+ cells of patients with AA, most significantly in patients with drug-induced AA (Calado et al., 2001). These findings point to a role of impaired P-gp function in determining predisposition to AA. However, the reason for reduced P-gp activity in this setting is elusive.

Strong evidence has emerged recently to demonstrate that P-gp expression is genetically determined. The C3435T transition of the MDR1 gene correlates with reduced P-gp expression and function in the duodenum and CD56+ natural killer (NK) cells (Hoffmeyer et al., 2000; Hitzl et al., 2001). To evaluate whether the MDR1 gene C3435T polymorphism is associated with the occurrence of AA, we studied 35 patients with acquired AA (median, 26 years; range, 2–67 years). Classification was very severe in five, severe in 20 and moderate in 10 patients; 20 AA cases were idiopathic. 13 were related to drugs or chemicals (anti-inflammatory drugs in two, pesticides in nine and solvents in two) and two were hepatitis-associated AA cases; 105 age-, sex- and ethnicity-matched healthy subjects (blood donors) from the same geographical area were studied as controls. Blood and bone marrow samples were collected after informed consent. DNA was extracted from peripheral leucocytes to genotype patients and controls for the MDR1 C3435T polymorphism using polymerase chain reaction (PCR) followed by DpnII enzyme digestion (Hoffmeyer et al., 2000). A technician, who was unaware of the sample status, performed the genotyping. Odds ratios (OR) as a measure of relative risks and 95% confidence intervals (CI 95) were calculated by standard methods.

Carrier frequency of 3435T was 62.8% amongst patients and 61% amongst controls, yielding an OR of 1.1 (CI 95, 0.5–2.4), or for heterozygosity was 1.3 (CI 95, 0.6–2.9); it was 0.6 (CI 95, 0.1–2.2) for mutant homozygosity (Table 1). When patients with drug-induced AA were analysed separately (n = 13), the overall OR linked to the mutant genotype was 0.7 (CI 95, 0.2–2.3). Heterozygous and mutant homozygous states yielded or of 0.5 (CI 95, 0.1–2.1) and 1.2 (CI 95, 0.2–6.3) respectively.

The MDR1 gene C3435T polymorphism is linked to reduced P-gp expression and function, and bone marrow CD34+ cells from 26 out of the 35 AA patients studied presented P-gp function (measured by the rhodamine 123 efflux assay) below the 25% percentile of previously studied normal controls (Calado et al., 2001). However, we failed to demonstrate a relationship between AA and C3435T. This lack of association may be explained by different reasons. First, the proportion of individuals developing AA after drug or chemical exposure is minimal compared with the prevalence of the C3435T polymorphism. It is possible that the decreased P-gp function observed in AA might be associated with other rare unknown MDR1 gene mutation(s) which might determine P-gp function in haematopoietic cells. Second, impaired P-gp function in AA might be the result of acquired rather than genetic factors. Testing whether defective P-gp expression is restricted to the
haematopoietic tissue may serve to address this question. Finally, the number of patients analysed here was relatively small making it difficult to definitively rule out an association between MDR1 C3435T polymorphism and AA.

In conclusion, although Pgp activity is decreased in AA patients, the MDR1 gene C3435T polymorphism does not seem to be a genetic risk factor for acquired drug-induced aplastic anaemia.

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REFERENCES


Keywords: aplastic anaemia, MDR1, P-glycoprotein, polymorphisms, risk factor.

INTERLEUKIN 8 IS NOT INVOLVED IN G-CSF-INDUCED PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Granulocyte colony-stimulating factor (G-CSF) is widely used to mobilize haematopoietic progenitor cells (HPCs) into the peripheral blood as a source of stem cells in autologous and allogeneic stem cell transplantation. However, the mechanisms of G-CSF-induced peripheral blood stem cell (PBSC) mobilization remains unclear. Interleukin 8 (IL-8) is
another cytokine that mobilizes HPCs into blood. Recently, the mechanism of IL-8-induced PBSC mobilization has been extensively studied. The release of matrix metalloproteinase-9 (MMP-9) from neutrophils by IL-8 induces HPCs mobilization by cleaving matrix molecules to which HPCs attach (Laterveer et al. 1995; Pruitt et al. 1999). In addition, Watanabe and colleagues (Watanabe et al. 1999) demonstrated a surge of endogenous IL-8 after G-CSF administration in normal donors, suggesting the critical role of IL-8 in G-CSF-induced PBSC mobilization. They therefore examined the kinetics of serum and plasma levels of IL-8 after G-CSF administration in 10 normal donors for allogeneic PBSC transplantation. After informed consent had been obtained, blood samples were collected by venepuncture before and during G-CSF administration at a dose of 10 μg/kg s.c. for 5 d. Plasma and serum IL-8 levels were measured using two specific enzyme-linked immunosorbent assay (ELISA) kits (TFB, Tokyo, Japan; R&D Systems, Minneapolis, MN) according to the manufacturers’ instructions. Each sample was analysed in duplicate, and the average value was used for calculation. The minimal detectable concentration was estimated as 10 pg/ml. In all donors except one, who obtained a total of 1.04 × 10⁶/kg CD34⁺ cells by three aphereses, more than 3 × 10⁶/kg CD34⁺ cells could be harvested by one to three aphereses. The serum and plasma levels of IL-8 before G-CSF administration in all samples were < 10 pg/ml, and were below the level of detection during G-CSF administration. These observations were in contrast to the report by Watanabe and colleagues (Watanabe et al. 1999), who demonstrated a surge of endogenous IL-8 (mean: 200 pg/ml) on days 5 and 6 of G-CSF administration. They used serum samples and measured the level of IL-8 using the same ELISA kit (TFB) as we used. We therefore measured serum as well as plasma samples, although the manufacturer’s instructions recommended using plasma samples. In addition, we confirmed our data using another ELISA kit (R&D Systems). The reason for the differences in serum IL-8 levels between the two studies remains unclear.

Michon and colleagues (Michon et al. 1998) demonstrated the increased IL-8 mRNA levels in peripheral blood cells after 24 h of G-CSF administration at a dose of 10 μg/kg s.c. after recovery of the previous chemotherapy cycle cytopenia in cancer patients. However, they could not detect circulating IL-8 in the serum samples, and speculated that IL-8 is either not synthesized/secreted or is rapidly trapped by IL-8 receptor on neutrophils. Recently, Carstanjen and colleagues (Carstanjen et al. 2001a) also demonstrated no increased plasma level of IL-8 after G-CSF administration in normal donors. These observations, together with ours, suggested that IL-8 was not involved in the G-CSF-induced PBSC mobilization in normal donors. Other mechanisms of PBSC mobilization by G-CSF intervened by other cytokines, including IL-6 or matrix metalloproteinases, are now under investigation (Carstanjen et al. 2001b).

REFERENCES
Carstanjen, N., Ulbrecht, A., Iacone, A., Regenfus, M. & Salama, A. (2001a) MMP-9 (Gelatinase B) is elevated during mobilization of peripheral blood stem cells by G-CSF. Experimental Hematology, 29 (Suppl. 1), 10.

Keywords: granulocyte colony-stimulating factor, peripheral blood stem cell, mobilization, interleukin 8.

EFFECTIVENESS OF RITUXIMAB FOR CHEMOTHERAPY-RESISTANT MULTIPLE TUMORAL B-LPD IN A HAEMOPOIETIC STEM CELL RECIPIENT

We read with great interest the recent article by Faye et al (2001). They described eight responders and four non-responders in a therapeutic trial of rituximab for post-transplant B-lymphoproliferative disorder (B-LPD) following haemopoietic stem cell transplantation in 12 paediatric patients. In their article, three out of the four
non-responders died, and non-responsiveness was associated with a high number of tumour sites involved (median 3–5), a mediastinal localization, and a significantly low CD4 cell count (median 4/μl).

The patient we report here was a 20-year-old man, who had been treated for aplastic anaemia for 4 years with methylprednisolone (mPSL)/cyclosporine A (CSA)/granulocyte colony-stimulating factor, and received an allogeneic bone marrow transplantation (BMT) from a human leucocyte antigen (HLA)-matched unrelated donor in November 1998. Epstein–Barr virus (EBV) status (donor/recipient) at the transplant was –/+ . The conditioning regimen was total lymphoid irradiation/cyclophosphamide/antithymocyte globulin with graft versus host disease (GVHD) prophylaxis of CSA/short-term methotrexate (MTX)/mPSL. The immediate post-transplant course was uneventful except for Grade I acute GVHD. At 2 months post transplant, the patient developed cytomegalovirus (CMV) retinitis, which was treated with gancyclovir and foscarnet. At day 150, he started complaining of abdominal discomfort in the right upper quadrant. At day 210 (June 1999), ultrasound and a computed tomography (CT) scan revealed bilateral adrenal tumours associated with a mass in the liver (Fig 1A) and another mass in the right kidney. A CT scan of the chest also disclosed multiple infiltrating shadows. At the time the patient was afebrile and had no gammanopathy. His CD4 count was 14–36/μl and CD20 count, 4–26/μl. Serum EBV load, determined by real-time polymerase chain reaction (Teramura et al., 2002), was 9 × 10³ (normal < 50) copies/ml. A needle biopsy of the liver mass revealed an EBER (EBV-encoded RNA)-positive B-LPD, which showed clonal rearrangement of immunoglobulin heavy chain gene. The tumours did not resolve by withdrawal of immunosuppressants; in addition, donor lymphocytes were unavailable from bank donations, so chemotherapy (one course of vincristine/cyclophosphamide/prednisolone, and another course of adriamycin/vincristine/cyclophosphamide/prednisolone) was administered. The tumours responded only temporarily, then exacerbated in November 1999 as multiple liver tumours (Fig 1B), with an sharp increase of serum EBV load up to 15 × 10⁴ copies/ml. The second liver biopsy at day 360 also showed an EBER-positive B-LPD. Four more courses of chemotherapy including MTX and cytosine arabinoside were not effective. Therefore, in April and May 2000, rituximab (375 mg/m²/weekly ×8 doses) was given. There was no prompt response, and para-aortic lymph nodes’ swelling indicated a new LPD 2 months later, associated with abdominal pain. Accordingly, a second course of rituximab (375 mg/m²/weekly ×8 doses) was administered in October–November 2000. Since then, the adrenal, hepatic and para-aortic masses gradually resolved over the following year, leaving only one regressing liver tumour, with undetectable EBV load in serum. During the period, no treatment was given except for high-titre anti-CMV-gamma-globulin replacement therapy. The patient has been doing well, with a 100% Karnofsky score as of November 2001 (36 months from the BMT and 28 months from the development of LPD).

In this case, the rituximab therapy, which began at 10 months from the development of B-LPD, was very effective, although the patient had chemotherapy-resistant multiple tumoral B-LPD. To achieve better control, a total of two courses was needed within a 4-month interval. A good response was reflected by monitoring the serum viral load, as reported by van Esser et al. (2001). A gradual, but steady, regressive effect on the tumours over a period of 1 year might have been due to a synergistic activity with the chemotherapy administered before the rituximab, as described in lymphoma therapy (Vose et al., 2001). Further evaluation of rituximab is necessary for multiple tumoral B-LPD in haemopoietic stem cell transplant recipients.

Fig 1. (A) The right adrenal tumour (arrowhead) and an intrahepatic mass at onset of LPD. (B) Large masses in the liver with right adrenal tumour (arrowhead) at exacerbation.

REFERENCES


**Keywords:** rituximab, post-transplant lymphoproliferative disorder, Epstein–Barr virus.

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**‘GLOVES AND SOCKS’ PAPULAR PURPURIC SYNDROME FOLLOWING PRIMARY INFECTION WITH PARVOVIRUS B19: A LINK BETWEEN DERMATOLOGISTS AND HEMATOLOGISTS**

‘Gloves and socks’ papular purpuric syndrome is characterized by pruritic and painful oedema and erythema localized to the hands and feet in a ‘gloves and socks’ distribution. It is often associated with oromucosal lesions with vesicular aphthous lesions localized to the hard and soft palate, pharynx and lips (Ruzicka et al., 1998; Saulsbury, 1998; Smith et al., 1998; Grilli et al., 1999; Martinez-Martinez & Maranon, 2000). Myalgia can be present (Ruzicka et al., 1998, Maranon, 2000). Fever, asthenia, anorexia, arthralgia and rash have been implicated in the pathogenesis of this disease (Ruzicka et al., 1998, Saulsbury, 1998; Smith et al., 1998; Grilli et al., 1999; Martinez-Martinez & Maranon, 2000). The frequent occurrence of lymph node enlargement and the detection of leucopenia and thrombocytopenia make this entity of special interest to haematologists. Several infectious agents, especially viruses, have been implicated in the pathogenesis of this disease (Ruzicka et al., 1998; Saulsbury, 1998; Smith et al., 1998; Grilli et al., 1999; Martinez-Martinez & Maranon, 2000). The frequent occurrence of ‘gloves and socks’ syndrome following a primary parvovirus B19 infection.

A 38-year-old human immunodeficiency virus-negative man presented in February 2001 with a 5-day history of pruritic oedema and erythema on the dorsum of both hands and feet, which subsequently progressed to involve the palms of his hands and the soles of his feet. The cutaneous lesions were symmetric and marginated on the wrists and ankles without mucosal involvement. The patient had intermittent fever (38.5°C) lasting 2 d, and bilateral axillary and inguinal lymph node enlargement was present. The liver and the spleen were not enlarged. The patient reported no insect bites, medication or travel abroad in the preceding months. Laboratory examination revealed leucopenia (lowest leucocyte count, 3.3 x 10⁹/L), with neutrophilia (neutrophils 71% and lymphocytes 12%) and thrombocytopenia (lowest platelet count, 75 x 10⁹/L), a minor reduction in haemoglobin level (from 14 to 12 g/dl) and a reticulocyte count of 1% (Fig 1). Erythrocyte sedimentation rate was 12 mm/h. Blood urea nitrogen, serum creatinine, electrolyte concentrations, total bilirubin, alkaline phosphatase, serum aspartate-transaminase, serum alanine-transaminase, coagulation tests and urinalysis were normal. Rheumatoid factor, antinuclear antibodies, anti-DNA anti-

bodies, and platelet-associated immunoglobulins (Ig) were not detected. Routine blood cultures for the most common bacterial, fungal and viral agents and serological and molecular studies for hepatitis, herpes, Coxsackie A and B and echoviruses were negative. The rise of IgM and IgG antibodies against parvovirus B19 was documented by enzyme-linked immunosorbent assay (ELISA) in serial serum samples collected during the acute and convalescent phases of the disease, proving seroconversion (Smith et al., 1998; Grilli et al., 1999) (Fig 1). The presence of B19 DNA sequences was detected in the serum samples from the acute phase, by polymerase chain reaction (Grilli et al., 1999) (Fig 1). The purpuric lesions and lymph node enlargement disappeared after 12 d, with a concomitant normalization of blood cell counts, without therapy.

The sudden onset of fever, dermatosis, lymphadenitis and cytopenia suggested the presence of an acute infectious disease. Seroconversion and high levels of viraemia were indicative of a recent primary infection with B19, whereas extensive microbiological tests failed to identify any other agents. Only 30 cases of ‘gloves and socks’ syndrome have so far been described, but none of these is in the haematology literature. Adult, often female, patients are affected: only two cases have been reported in children. Four cases have been associated with cytomegalovirus (Smith et al., 1998), Coxsackie B6 (Smith et al., 1998), measles (Smith et al., 1998) and human herpesvirus 6 infection (Ruzicka et al., 1998), respectively. A total of 17 cases has been associated with B19 infection, whereas no infectious agents were searched for, or identified, in the remainder (Saulsbury, 1998; Smith et al., 1998; Grilli et al., 1999; Martinez-Martinez & Maranon, 2000). B19 is the aetiological agent of erythema infectiosum, has tropism for erythroid precursors in bone marrow and may cause either transient aplastic crises in immunocompromised hosts or thrombocytopenia in sporadic cases. B19 infection must be recognized as a common cause for the ‘gloves and socks’ syndrome. An atypical aspect of primary B19 infection in this and previously reported cases is the almost constant preservation of erythroid lineage in the bone marrow.
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Department of Oncology and Hematology, Section of Hematology, University of Modena and Reggio Emilia, Modena, Italy.

E-mail: gtorelli@unimo.it

REFERENCES


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Fig 1. Changes in the platelet and white cell counts, haemoglobin level, serum parvovirus B19 DNA and anti parvovirus B19 antibodies during acute and convalescent phases of the disease. Values for IgG and IgM were considered positive if they were both above the cut-off optical density value of 0.9.