## Dramatic response to single-agent rituximab in a patient with intravascular lymphoma

*To the Editor:* Intravascular lymphoma (IVL) is an extremely rare subtype of diffuse large B-cell lymphoma, characterized by the occlusion of small blood vessels with clonal lymphoid cells [1]. Neoplastic cells are commonly of B-cell origin, although rare cases of T or NK-cells have been reported [2–4]. The mechanism for the peculiar blood vessel tropism of IVL is not known. Ponzoni et al. [5] demonstrated defects in the surface expression of adhesion molecules ($\beta_1$ integrin and I-CAM) responsible for intravascular migration of lymphocytes in IVL, which could be associated with this characteristic distribution pattern. Anthracycline-based chemotherapy is considered the standard front-line treatment, however, its utility is limited because of relatively short remission durations and poor performance status at presentation [1,2]. Rituximab (Rituxan, Genentech, San Francisco, CA) is an attractive treatment option in IVL because of the intravascular location of brightly CD20 positive lymphoid cells. We report here a case of IVL in a 63-year-old patient who had a dramatic sustained remission to planned treatment with single-agent rituximab without anthracycline-based chemotherapy.

A 63-year-old white male presented with fevers (103°F) and a nonproductive cough. His past medical history was significant for diabetes, hypothyroidism, hyperlipidemia, and celiac disease with associated iron deficiency anemia. On two prior occasions, he had suffered similar symptoms but a thorough workup of his symptoms including blood cultures, hepatitis profile, HIV testing, radiographic imaging, bone marrow biopsy, and lumbar puncture etc., recovered only with a diagnosis of FUO. On the most recent occasion, his physical examination was unremarkable. Laboratory studies showed mild anemia, platelet count of 296 $\times$ $10^9$/l, an erythrocyte sedimentation rate of >140 mm/hr, and lactate dehydrogenase (LDH) of 788 U/l (normal range 94–172 U/l). CT-scans of chest, abdomen, and pelvis were normal.

High fevers persisted despite empiric antibiotics. A workup of noninfectious causes of fever including collagen-vascular and autoimmune diseases was negative. The patient's performance status continued to decline. Two weeks after presentation he started to develop a truncal maculopapular rash (Fig. 1). His rash spread to include the proximal aspects of his extremities. A skin biopsy was performed which revealed dermal blood vessel lumen occlusion by proliferation of large, atypical lymphoid cells with high nuclear/cytoplasmic ratio, prominent nucleoli, and fine chromatin lymphoma (Fig. 2). As shown in Fig. 3, immunohistochemical stain for CD20 reveals strong membrane positivity of the atypical lymphocytes in the small dermal vessels. In addition to CD20, the atypical lymphoid cells were also positive for CD45, CD19, CD22, CD79a but not CD3, CD5, CD30, and AE1/AE3 establishing the diagnosis of IVL. Given the patient's poor performance status, the decision was made to treat with single-agent rituximab (375 mg/m$^2$ weekly infusion). Interestingly, after just 2-weekly doses, his rash dramatically resolved and his fevers and cough completely abated. His LDH normalized and his performance status dramatically improved to near baseline. He is currently in complete remission for 9 months after receiving 8-weekly rituximab doses.

IVL is an exceedingly rare subtype of diffused large B-cell lymphoma with an estimated incidence of less than one new case per million populations [6]. Patients often present with skin rash, focal neurological deficits, or FUO. LDH is elevated in majority of the cases. Diagnosis is established by demonstrating collection of large, atypical lymphoid cells in the lumen of blood vessels. Immunohistochemically these cells are positive for CD19, CD20, CD22, CD79a and surface immunoglobulin. Immunoglobulin genes are clonally rearranged [2].

Although, response rates to chemotherapy as high as 83% have been reported [7], time to progression is often short and median survival is typically less than 12 months [1,7]. Small retrospective series of IVL patients treated with CHOP have reported response rate of 43% and median survival of 5months [2,7]. Currently, immunochemotherapy with the combination of CHOP plus rituximab is considered the gold standard for the treatment of diffuse large B-cell lymphoma [6]. Given the typically bright expression of CD20 and the propensity of neoplastic cells to remain in the intravascular space, rituximab is

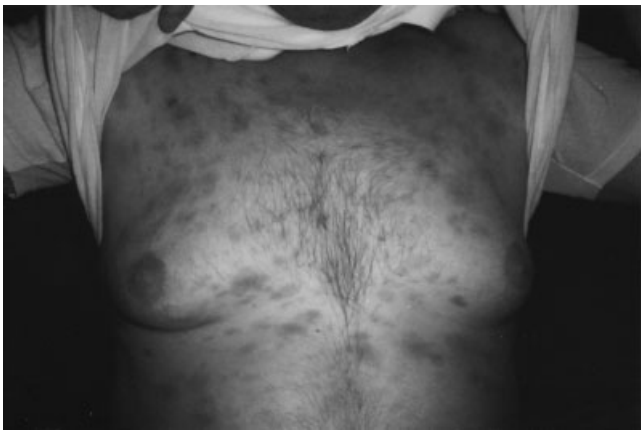

**Fig. 1. Violaceous truncal maculopapular rash.**

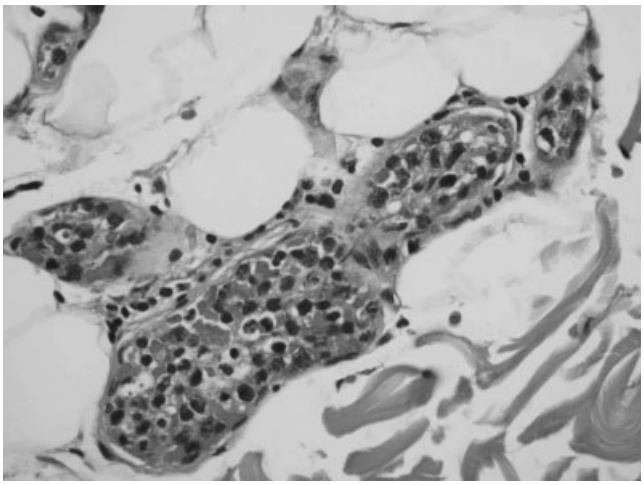

**Fig. 2. Dermal blood vessels are distended and occluded by cluster of atypical lymphoid cell. Hematoxylin and eosin stain (×100).**

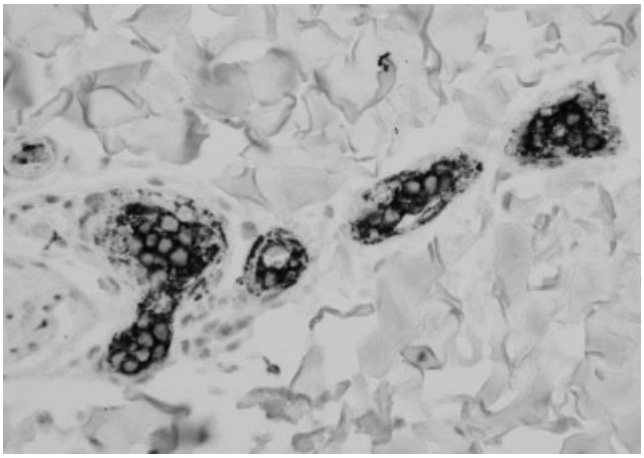

**Fig. 3. Atypical lymphoid cells with strongly positive staining for CD20.**

an attractive treatment option. In fact, one group has reported an ongoing remission of over 3 years to rituximab therapy for IVL after the patient experienced severe toxicity to CHOP-based treatment [2]. A handful of other reports have documented responses to therapies incorporating rituximab [2,6]; however, ours is the first case to our knowledge to report such a dramatic response to planned first-line, single-agent rituximab therapy. Although rituximab is generally well tolerated, rare cases of acute respiratory distress syndrome requiring mechanical ventilation following rituximab administration in IVL have been reported [8]. IVL patients with disease limited to skin (cutaneous variant), have a better prognosis compared with ones with disseminated disease. Single-agent rituximab therapy appears to be a potential therapeutic option for cutaneous variant of IVL. Similarly, rituximab may be considered for IVL patients with advanced age, poor performance status, and those with contraindications or intolerant to standard combination chemotherapy.

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*DOI: 10.1002/ajh.21005*

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## Therapeutic use of Rituximab for sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)

*To the Editor:* Sinus histiocytosis with massive lymphadenopathy (SHML), also recognized as Rosai-Dorfman disease (RDD), is characterized by a nonmalignant proliferation of distinctive hystiocytic/phagocytic cells within lymph node sinuses and extranodal lymphatics. Multiple treatment modalities have been employed in small numbers of patients without objective measures of response [1]. One course of the anti-CD20 antibody Rituximab, however, led to successful treatment of SHML in a prior case report [2]. Here we report the immunotherapeutic use of repeated Rituximab dosing in a patient with multirelapsing SHML.

A 29-year-old woman developed recurrent upper respiratory symptoms and multiple enlarged cervical lymph nodes after delivering her second child. An excisional lymph node biopsy demonstrated dilated cortical and medullary sinuses filled with large histiocytes having round-vesicular nuclei and abundant pale vacuolated cytoplasm (Fig. 1A). Many of the histiocytes contained intact cytoplasmic lymphocytes and less often neutrophils, plasma cells, and erythrocytes consistent with a process of lymphophagocytosis or emperipolesis (Fig. 1B) [3]. The immunohistochemical findings showed the histiocytes to express S100 and CD68, without significant CD1a expression consistent with a diagnosis of SHML. In addition, the lymph node was composed of a mixture of B (CD20 positive) and T (CD3 positive) lymphocytes with many of the intracytoplasmic lymphocytes identified as CD20 positive B-lymphocytes (Fig. 1b).

The patient's symptoms increased with development of debilitating joint pain and profound fatigue requiring therapy. Based on the lack of degradation of the observed CD20 lymphocytes housed within the histiocytes and worsening symptoms, an empiric course of Rituximab (375 mg/m$^2$ weekly for 4 consecutive weeks) was administered. The patient had complete resolution

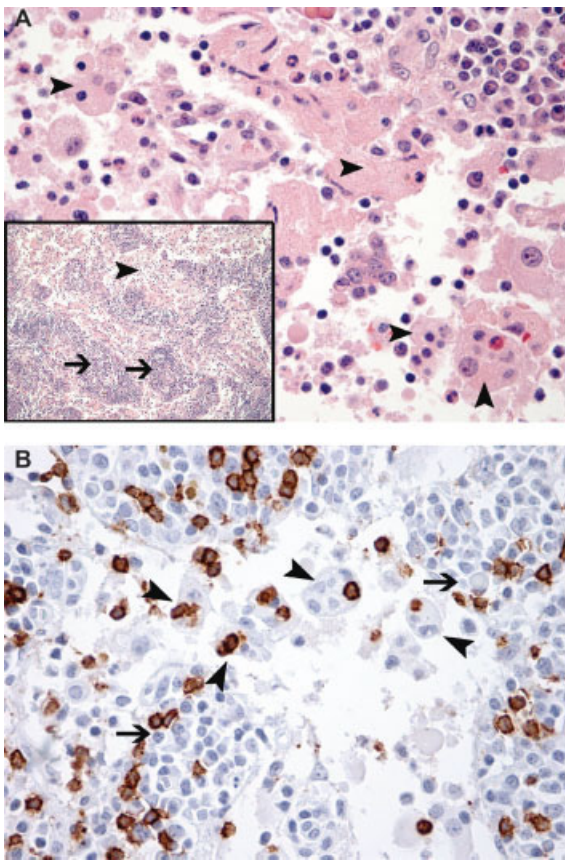


**Fig. 1. Histologic features of sinus histiocytosis with massive lymphadenopathy. (A) The lymph node is enlarged by massively dilated sinuses filled with large atypical histiocytes (arrowheads) containing cytoplasmic lymphocytes, plasma cells, neutrophils, and eosinophils. The medullary cords (long arrows) contain large collections of plasma cells and lymphocytes with reactive follicles. (B) Anti-CD20 immunohistochemical studies demonstrating CD20 positive B-lymphocytes present in the cytoplasm of large atypical histiocytes (arrowheads) and scattered throughout the medullary cords (long arrow). Note that not all of the intracytoplasmic cells are CD20-positive lymphocytes. Many plasma cells are present in the medullary cords with a rare Russell body (large arrow). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

of the lymphadenopathy and normalization of her symptoms within 4 weeks of completing therapy. However, after completion of her third pregnancy 18 months later, severe arthralgias, respiratory symptoms and 3- to 4-cm adenopathy returned. Retreatment with Rituximab again led to resolution of the patient's symptoms and adenopathy. The patient again relapsed 6 months later and achieved a complete remission with a third course of Rituximab. In an attempt to extend the duration of response, 1 dose (375 mg/m$^2$) of Rituximab was delivered every 3 months over the next year while in remission with maintenance of response to date.

The true pathogenic mechanism responsible for Rituximab antibody-mediated response is unclear. Since the anti-CD20 antibody could not target intracellular CD20+ lymphocytes harbored inside histiocytes, the effect must depend on reducing the extracellular pool of CD20+ lymphocytes or tagging extracellular lymphocytes prior to cytoplasmic entry. Cytoreduction of the CD20+ lymphocytes may decrease the available targets for emperipolesis. Alternatively, the effect of Rituximab may be due to inhibition of immunomodulatory signals or indirect targeting of the precursor cells supporting the plasmacytic infiltrate and associated hypergammaglobulinemia [4]. In support of this hypothesis, similarities are shared between autoimmune lymphoproliferative syndrome (ALPS) and SHML; SHML might arise from similar defects in the Fas-mediated apoptotic pathway present in ALPS [5]. Treatment with Rituximab may have served to target apoptosis in defective lymphocytes. Overall, this immunotherapeutic modality may contribute to a better understanding of the pathophysiologic mechanisms, as well as improved therapies, for this entity.

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*DOI: 10.1002/ajh.21024*

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## Immune thrombocytopenia from vancomycin in orthopedic cement

*To the Editor:* We report a case of drug-induced thrombocytopenia through a previously unreported mode of exposure.

A 56-year-old female presented for removal of an infected knee prosthesis. At initial implantation in August 2005, postoperative bleeding occurred within 6 hr, associated with a platelet count drop to 2,000/$\mu$L from 160,000/$\mu$L. She was given platelets, red blood cells, and methylprednisolone. The platelet count recovered to 96,000/$\mu$L in 4 days and then normalized. Marrow biopsy demonstrated normal histology. The thrombocytopenia was presumed due to prophylactic intravenous vancomycin.

She suffered refractory infections of the prosthesis and required removal. In January 2006, the patient underwent removal of the knee prosthesis and insertion of interspace medium antibiotic-containing prosthesis components fixed with Palacos® cement laced with antibiotics. Intravenous vancomycin was avoided. She had no heparin exposure. Within hours postoperatively the patient developed significant bleeding from the operative site, ecchymoses and petechiae on her extremities, trunk, and face. Four hours postoperatively, her platelet count was less than 10,000/$\mu$L down from 328,000/$\mu$L. She received platelets and red blood cells. She was given intravenous immunoglobulin for 2 days beginning within 12 hr of bleeding onset. Her platelet count was less than 10,000/$\mu$L for 2 days; on the third postoperative day it rose to 14,000/$\mu$L and was 280,000/$\mu$L by postoperative day 7.

Records from her August 2005 surgery were compared carefully with records of her January 2006 procedure. Vancomycin was noted in her 2005 records as the suspected culprit. Upon initial comparison, only three drugs were used with both surgeries—fentanyl, succinylcholine, and lidocaine. Later, the medical student noted that there had been 4 g of vancomycin powder mixed into the Palacos cement. Platelet antibody evaluation was strongly positive for vancomycin drug-dependent platelet antibodies and negative with fentanyl and succinylcholine. In March 2006, the spacer and all traces of antibiotic-laced cement were removed prior to insertion of a new prosthesis without recurrence of severe thrombocytopenia.

Thrombocytopenia is not an uncommon complication of drug administration [1,2]. Most exposures reported are oral, intravenous, or intramuscular [3]. Heparin-induced thrombocytopenia has been noted to occur or be exacerbated by heparin-coated intravenous catheters [4]. This case demonstrates that exposures by unusual routes can lead to serious consequences. It also demonstrates the value of medical student sleuths to find clues that unlock patient mysteries!

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*DOI: 10.1002/ajh.20916*

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## Aprotinin-associated hemolytic thrombotic microangiopathy in a patient with acute myelogenous leukemia (AML) and systemic coagulopathy

*To the Editor:* The pathophysiology of hemostatic perturbances in AML is complex and may be severely confounded by leukemic cell over-expression of procoagulant (i.e. tissue factor [TF]) and/or fibrinolytic factors (i.e. urokinase-type plasminogen activator [uPA] and its receptor [uPAR]) [1–3]. Early and

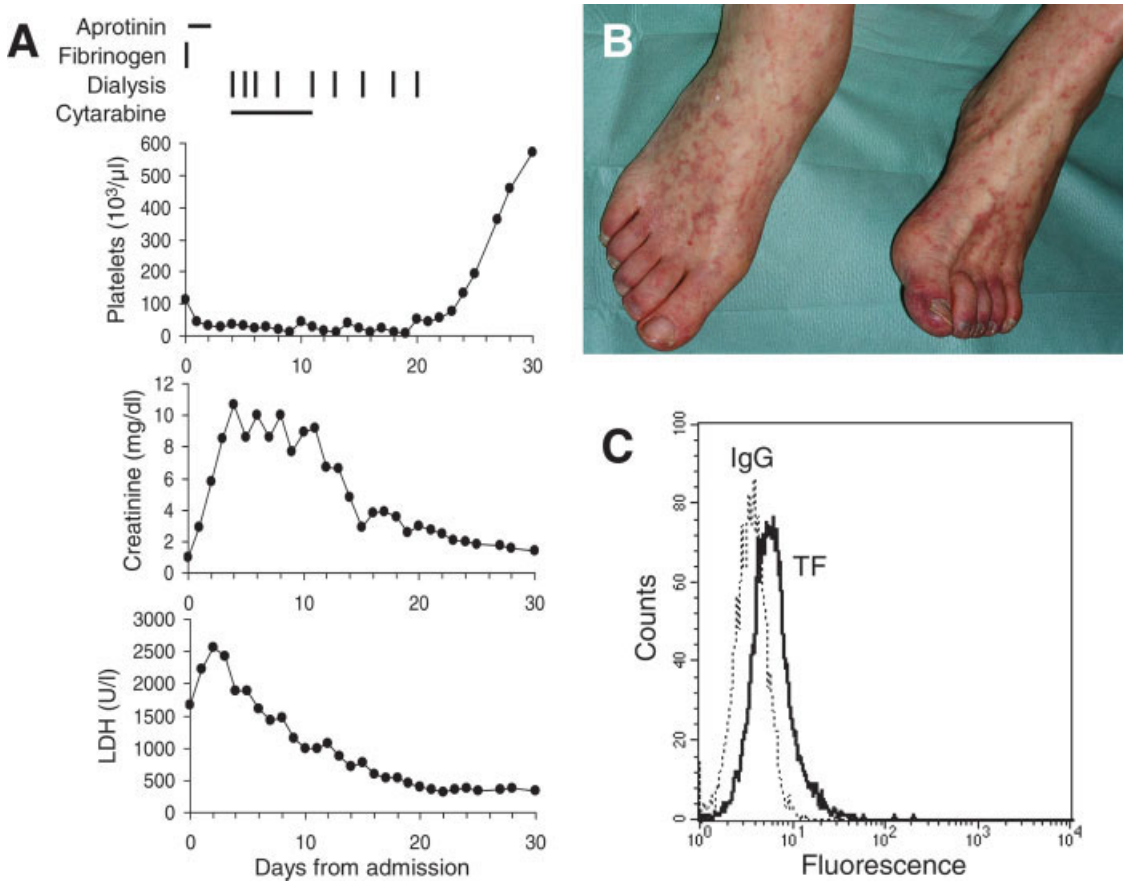


**Fig. 1. (A) Changes in whole blood platelet count and plasma levels of creatinine and lactate dehydrogenase (LDH). (B) Livedo reticularis of the feet. (C) Single-color flow cytometric analysis of TF antigen expression on peripheral monoblasts. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

correct diagnosis of systemic coagulopathies, however, is mandatory to prevent potentially life-threatening bleeding in patients with AML.

A 69-year-old man with newly diagnosed acute monocytic leukemia presented with spontaneous cutaneous hematomas and sustained bleeding from venous puncture sites due to severe hypofibrinogenemia of 0.3 g/l (normal range, 1.8–3.5 g/l). The prothrombin time was prolonged with an INR of 2.51 (0.85–1.25), and the activated partial thromboplastin and thrombin time were prolonged to 48 sec (25–38 sec) and 62 sec (16–22 sec), respectively. The plasma D-dimer level was 3333 µg/l (50–190 µg/l), and the plasma antithrombin activity was 87% (70–130%). Concentrations of whole blood hemoglobin, leukocytes, and platelets were 15 g/l, 5.5 $\times$ $10^3$/µl, and 114 $\times$ $10^3$/µl, respectively. To limit excessive fibrino(geno)lysis and prevent potentially fatal hemorrhage, the patient received an intravenous bolus of 1.5 million units of aprotinin (Trasylol™) followed by a continuous infusion of 200,000 units/hr. In addition, a total of 4 g of human fibrinogen were substituted. Within 48 hr thereafter, the patient developed anuria, progressive thrombocytopenia, and livedo reticularis of both feet with bluish discoloration of the left toes indicating cutaneous microvascular thrombosis (Fig. 1A,B). Bilateral color-coded duplex ultrasonography revealed diminished perfusion of the kidney parenchyma consistent with small vessel occlusion. Up to 26‰ (<5‰) of fragmented erythrocytes were seen on peripheral blood smear. There was laboratory evidence of intravascular hemolysis as indicated by hyperbilirubinemia, a further increase in LDH levels (Fig. 1A), and a decrease in plasma haptoglobin. The plasma activity of the von Willebrand factor-cleaving metalloproteinase (ADAMTS13), a complete deficiency (<5%) of which is characteristic of thrombotic-thrombocytopenic purpura [4], was 39% (30–120%). The patient underwent hemodialysis and received a seven-day course of continuous intravenous cytarabine (100 mg/m$^2$/24 hr) together with unfractionated heparin (7500 IU/24 hr). This treatment resulted in significant improvement of kidney function, resolution of laboratory, and clinical evidence of intravascular hemolysis and coagulopathy, and complete hematological AML remission. While receiving a cytoreductive maintenance therapy with hydroxyurea, the patient was well and free of disease-related symptoms at three months of follow-up.

A more detailed laboratory assessment of the initial plasma sample revealed evidence of disseminated intravascular coagulation (DIC) with consumption of numerous clotting factors, hyperfibrino(geno)lysis, and exhaustion of the endogenous anti-fibrinolytic system. Furthermore, using flow cytometry and a modified prothrombin time assay [5], we could demonstrate significant expression of TF antigen (Fig. 1C) and procoagulant activity [not shown] on peripheral monoblasts. In contrast, while clearly positive for uPAR, leukemic cells did not over-express uPA [not shown].

In this AML patient with a clinically relevant bleeding diathesis due to TF-driven DIC, administration of aprotinin and fibrinogen resulted in a thrombotic microangiopathy reminiscent of the classic childhood hemolytic-uremic syndrome. Therefore, caution is warranted when contemplating anti-fibrinolytic treatment strategies in bleeding AML patients with suspected hyperfibrino-(geno)lysis.

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## Treatment of systemic mastocytosis with denileukin diftitox

Patients with systemic mastocytosis (SM) have a poor quality-of-life owing to abnormal proliferation of neoplastic mast cells in various organs causing symptoms related to release of mast cell mediators [1]. In addition, the life expectancy of patients with aggressive forms of SM is significantly reduced, which underscores the need for new effective therapeutic approaches. CD25, the alpha subunit of the interleukin-2 receptor (IL-2R), is aberrantly expressed almost universally on the membrane of neoplastic mast cells [2], which makes it an attractive therapeutic target. Denileukin diftitox ($DAB_{389}$IL-2, Ontak™) is a DNA-derived cytotoxic protein composed of the amino acid sequences of diphtheria toxin fragments A and B followed by the sequences for IL-2 [3]. The IL-2 moiety of the molecule directs the fusion protein to cells bearing the IL-2 receptor. Upon internalization, Ontak™ is proteolytically cleaved, which releases the diphtheria toxin that inhibits intracellular protein synthesis, which causes cytotoxicity. Accordingly, we designed a pilot trial in which Ontak™ was administered to patients with SM, initially at 9 $\mu$g/kg/day intravenously on days 1 through 5 of a 21-day cycle. This dose was increased after 3 months to 18 $\mu$g/kg/day in the absence of response and toxic side effects. Eight symptomatic patients with SM (despite optimal supportive care) received therapy with Ontak™. Their median age was 58 years (range 41–67) and time from diagnosis to Ontak™ therapy 39 months (range 1–158). At Ontak™ start, the median percentage of bone marrow mast cells was 20% (range 5–80%). For three patients, Ontak™ represented the first therapy for SM whereas five others had previously failed imatinib ($n = 4$), dasatinib ($n = 1$), or cladribine ($n = 1$). On physical examination, one patient, who had undergone splenectomy, had hepatomegaly and other had 4-cm splenomegaly prior to Ontak™ start. The FIP1L1-PDGFR$\alpha$ transcript was negative by PCR analysis in five assessable patients. Patients received a median of six cycles of Ontak™ (range 3–12). No responses have been observed among any of the patients, including two who had their dose increased to 18 $\mu$g/kg/day for three cycles. No significant difference was found between pre- and post-Ontak™ therapy regarding percentage of bone marrow mast cells ($P = 0.60$) or serum tryptase levels ($P = 0.91$) in any patient. Therapy was well tolerated with only three instances of grade three toxicity including bone pain, hypophosphatemia, and lower gastrointestinal bleed (all unlikely related to therapy).

Cells expressing all three subunits of the IL-2R, CD25 (alpha), CD122 (beta), and CD132 (gamma), have the greatest affinity for Ontak™ [4]. The presence of the intermediate-(beta + gamma) or high-affinity (alpha + beta + gamma) IL-2R is required for adequate internalization [5]. It is likely that the lack of activity of Ontak™ in this study relates to the absence of coexpression of CD25 with CD122 or CD132 IL-2R subunits by neoplastic mast cells, which would impair the efficient internalization of the fusion toxin [5]. In conclusion, Ontak™ at the dose schedule used in this study is well tolerated but is not clinically effective in SM. Alternative specific anti-CD25 agents deserve a trial in this disorder.

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*DOI: 10.1002/ajh.21013*

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## A single nucleotide polymorphism in the promoter region of the *NCF-2* gene

*To the Editor:* The clinical relevance of the NADPH oxidase is demonstrated by chronic granulomatous disease (CGD), an inherited disorder characterized by recurrent infections due to a failure of phagocytic leukocytes to produce reactive oxygen species. Genetic alterations in $p67^{PHOX}$ gene result in an autosomal recessive form of CGD [1]. We have previously described different single nucleotide changes in some $p67^{PHOX}$-deficient patients that apparently were not responsible for the CGD phenotype, but they could modify in some extend gene expression [2]. One of those was a C→T transition at position-23 of the 5′ regulatory region of the *NCF-2* gene. The aim of this work was to investigate the frequency of this single nucleotide substitution located in the promoter region of the *NCF-2* gene, as well as its consequences on gene expression.

Sixty-seven of a 100 healthy subjects group were homozygous for C, 32 were heterozygous and just one homozygous for T was detected. The frequency for the T allele was 17%, confirming a polymorphism of the *NCF-2* gene. This group was found to be in Hardy–Weinberg equilibrium ($0.50 > P > 0.30$) [3] (Figure 1). This nucleotide substitution is the first report in the promoter region of *NCF-2*. The nucleotide sequence has been submitted to the GenBank data bank with accession number DQ662964.

Leukocytes form both homozygous and heterozygous individuals revealed normal respiratory burst activity as assessed by the dihydrorhodamine assay. The median positivity of the test was 99.4% cells of the C/C individuals, 98% cells of the C/T individuals, and 99.2% cells of the T/T individual. There was no statistical difference among these groups ($P > 0.05$, Kruskal–Wallis test).

To investigate whether this substitution had an effect on gene expression, constructs containing mutated sequences were prepared, and transfected into HL60 cells. No differences were observed in the luciferase activity between the allele carrying a T at position-23 of *NCF-2* gene promoter and the allele with a C in that position ($P = 0.1049$, $n = 16$, Kruskal–Wallis test).

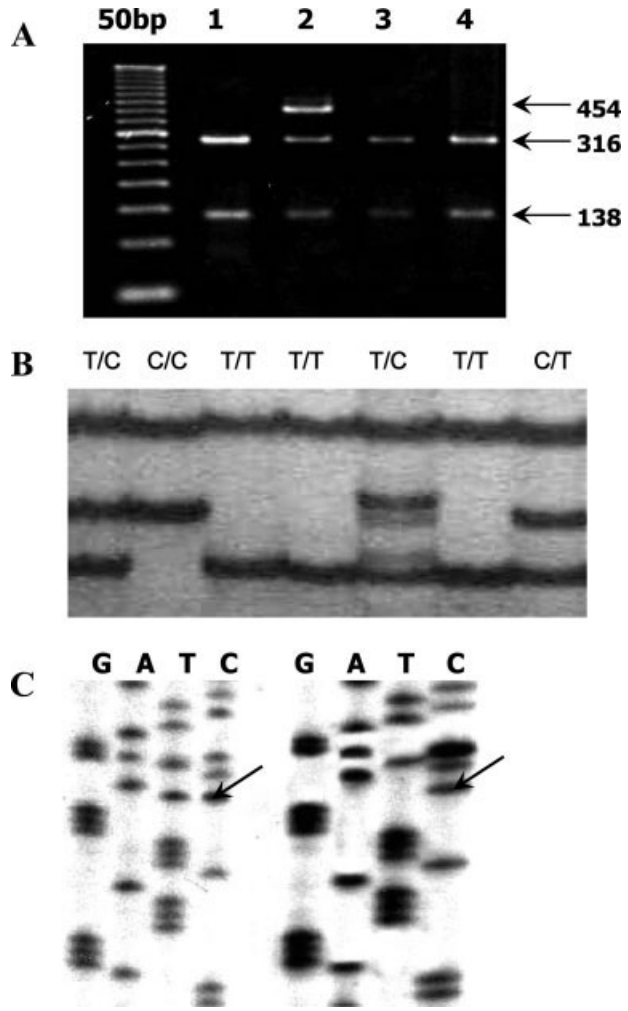


**Fig. 1. (A) Agarose gel electrophoresis of PCR fragments from the 5′ region of the *NCF-2* gene digested with Mae III. Lanes 1, 3 and 4 show digestion patterns of subjects homozygous for C in position-23; lane 2 shows a heterozygous subject (C/T). (B) SSCP analysis of PCR fragments from the 5′ region of the *NCF-2* gene in 9 healthy subjects. The concordance of these migration profiles with different genotypes in position-23 of the promoter was confirmed by digestion with Mae III and DNA sequencing. (C) DNA sequence of the 5′ region of the *NCF-2* gene in two healthy subjects presenting different genotypes. Arrows indicate the nucleotides present in position-23.**

The transcription of the *NCF-2* gene is regulated by several factors, including PU.I, IRF-1, ICSBP, and CBP [4]. Li et al. observed that mutations in consensus sequences including AP1, SP1, PU1, or the HAF-1 complex reduced the promoter activity, showing that this segment preserves the principal elements necessary for regulating transcription in this myeloid cell line [5].

The polymorphism that we here analyzed is not located in any known regulatory sequence of the *NCF-2* gene promoter, and this may be the reason for not affecting RNA expression. We conclude that the $23^{C/T}$ substitution is a polymorphism of the *NCF-2* gene, and affected individuals show normal respiratory burst activity and $p67^{PHOX}$ gene expression.

## Acknowledgments

The authors thank Sen-Lin Li and Robert A. Clark from the Department of Medicine, University of Texas Health Science Center that donated the pGL3 Luciferase Vector with clone 985-4.

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*Published online 21 August 2007 in Wiley InterScience (www.interscience.wiley.com).*
*DOI: 10.1002/ajh.21015*

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