

AUTOIMMUNE MEDIATED THROMBOCYTOPENIA ASSOCIATED WITH THE USE OF INTERFERON- α IN CHRONIC MYELOID LEUKEMIA

Eliana Zuffa, Nicola Vianelli, Giovanni Martinelli, Pierluigi Tazzari,* Michele Cavo, Sante Tura

*Institute of Hematology Seràgnoli, University of Bologna; *Service of Immunohematology and Transfusion, Policlinic S. Orsola, Bologna, Italy*

ABSTRACT

We report on two patients with Ph⁺ chronic myeloid leukemia (CML) in chronic phase who developed severe thrombocytopenia during treatment with interferon- α 2A (IFN- α 2A). In both cases, we detected the presence of platelet-associated antibodies (PAIg) by autoimmune flow cytometry. We postulated an immune mediated platelet destruction mechanism; corticosteroid therapy was employed and interferon therapy was withdrawn, resulting in an increase in platelet count and a reduction of PAIg. Our observation reports the detection of PAIg associated with IFN- α 2A therapy in CML and suggests that this immunomodulant drug could induce thrombocytopenia through a mechanism other than antiproliferation.

Key words: chronic myeloid leukemia, interferon- α 2A, thrombocytopenia, PAIg

Interferon- α 2A (IFN- α 2A) is effective in chronic myeloid leukemia (CML), providing hematological control in around 70% of patients and achieving significant cytogenetic improvement in up to one third.^{1,2} The mechanism of action of interferon appears to be non-selective inhibition of both normal and leukemic committed stem cells and an effect on the marrow stroma has also been postulated.^{3,4} How interferon exerts its effects at the molecular level is still not understood. Interferon-induced suppression of the Ph⁺ clone and the re-emergence of Ph-negative hematopoiesis may be explained by the observation that cells expressing P210 bcr/abl are more sensitive to growth inhibition by interferon than their normal counterparts.³ Another possible explanation is that leukemic cell clones may be attacked by cytotoxic CD8 lymphocytes that are activated by the expression of the HLA class I allele induced by an interferon responsive gene. These reported effects of interferon in CML patients may be due to enhancement of the immune response

related to an increase in HLA class I expression, but could also induce HLA restricted autoimmune disease.⁵

We report two cases of severe autoimmune thrombocytopenia with the presence of platelet-associated antibodies (PAIg) detected by flow cytometry.⁶

Patients and Methods

Case 1

CML was diagnosed in a 41-year-old man in October 1988. Hydroxyurea therapy was given until January 1989 when IFN- α 2A (Roferon-A) was started: 3 MU/day for 2 weeks, then 6 MU/day for 2 weeks, then 9 MU daily for 1 year. In February 1990, the patient showed a complete hematological response and a major karyotypic conversion. In September 1990, hematopoietic cell have harvested from bone marrow and in March 1991 the patient was submitted to autologous bone marrow transplant (ABMT). Two

Correspondence: Dr. E. Zuffa, Istituto di Ematologia "Seràgnoli", Policlinico S. Orsola, via Massarenti 9, 40138 Bologna, Italy. Tel. international +39.51.6363680. Fax international +39.51.398973.

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months later ABMT, karyotypic conversion was complete and only in November, at the reappearance of Ph⁺ positive metaphases, was treatment with IFN- α 2A resumed, at a dose of 6 MU/day for 8 months, then reduced to 3 MU daily because of liver toxicity. Until February 1993 the patient presented normal hematologic values and complete cytogenetic conversion, and continued treatment with IFN- α 2A at the same dose. In April 1993 he developed severe thrombocytopenia ($2 \times 10^9/L$) and a bone marrow aspirate showed a normal number of megakaryocytes without cytogenetic evidence of Ph⁺ metaphases; IFN- α 2A therapy was stopped and deflazacort was started at a dose of 2 mg/kg. After 15 days a normal platelet count was recovered, steroid treatment was reduced to 6 mg/day; IFN- α 2A was resumed (3 MU daily) until October 1993, when platelets decreased to $40 \times 10^9/L$. IFN- α 2A was stopped, deflazacort was increased to 90 mg/day and platelets increased to $201 \times 10^9/L$. In March 1994 therapy with IFN-n1 (Wellferon) was introduced (3 MU daily) and after 4 months platelet count again decreased to $40 \times 10^9/L$, with positivity for PAIg (direct assay); cytogenetic assessment showed a major karyotypic conversion. IFN-n1 was stopped, no steroid therapy was given and the platelets returned to normal values. In October 1994 PAIg were negative and natural IFN- α (alphaferone) was begun at a dose of 3 MU/day. In the last 12 months the patient has been in good clinical condition, with a normal platelet count, negative PAIg (direct and indirect assays) and a major karyotypic conversion. He is still on treatment with natural IFN- α 3 MU daily.

Case 2

A 28-year-old man developed CML Ph⁺ in May 1994. IFN- α 2A (Roferon-A) was started at a dose of 3 MU daily for 15 days then 4.5 MU daily; in June 1994 the dose was increased to 9 MU daily and the patient obtained a complete hematologic response after 3 months of treatment. In October 1994 he developed thrombocytopenia ($57 \times 10^9/L$) and IFN- α 2A was reduced to 4.5 MU/day. Nevertheless, thrombocytopenia progressed and IFN- α 2A was discontinued in December 1994 because the platelet count had

fallen to $2 \times 10^9/L$, with normal Hb and normal WBC count. Serum PAIg were detected (direct assay) and bone marrow aspirate showed an increase in the megakaryocyte number with normal cellularity. Treatment with prednisolone 1 mg/kg/day was started and after two weeks platelets increased to $102 \times 10^9/L$. PAIg remained positive. The patient had prednisolone reduced to 0.2 mg/kg/day and resumed IFN- α 2A 3 MU daily. In February 1995 hematologic values were normal, positivity for PAIg was decreased and cytogenetic assessment showed a major karyotypic response. The patient continued treatment with steroids and low doses of IFN- α 2A (3 MU daily). In March 1995 PAIg were negative. In April 1995 the platelet count fell to $42 \times 10^9/L$ with PAIg negativity. The prednisolone dose was increased to 0.5 mg/kg/day and IFN- α 2A was substituted with IFN-n1 (Wellferon) at a dose of 3 MU/day. After 2 months IFN-n1 was discontinued because of further platelet reduction ($18 \times 10^9/L$) and reappearance of PAIg. Azathioprine (100mg/day) was added to prednisolone (0.5 mg/kg/day) in June 1995. This therapy induced a normalization of platelet count within three months with maintenance of slight PAIg positivity. In September 1995 treatment was withdrawn and the patient currently shows a normal platelet number and serum PAIg positivity.

Discussion

The effects of interferon on hematopoietic progenitors and marrow stroma cells include growth inhibition, modulation of adhesion molecule expression and release of growth-regulating cytokines and receptors.³ A reduction in platelets has been reported in the majority of patients during interferon treatment due to a direct antiproliferative effect.⁷ Furthermore, interferon is capable of enhancing the expression of major histocompatibility antigens and of activating lymphocytes that mediate antigen-specific and nonspecific cytotoxicity.^{5,8} These and possibly other as yet undefined biologic features may induce the activation of immunological disorders. A recent report has described the development of symptomatic autoimmune thyroid dis-

eases in 4 (7.4%) out of 54 patients in chronic treatment with recombinant α -IFNs.⁹ However, the use of IFN- α 2A has been advocated in the treatment of immune thrombocytopenic purpura, even though its immunomodulator properties could exacerbate diseases that occur as a result of autoimmunity,^{10,11} such as autoimmune hemolytic anemia, thrombocytopenic purpura, hypothyroidism and thyrotoxicosis.

The two cases described underline the problem of severe autoimmune thrombocytopenias experienced with the use of IFN- α 2A. We observed an increased number of megakaryocytes in the bone marrow; furthermore, antiplatelet antibodies were detected. The platelet number recovered after interruption of IFN- α 2A and introduction of steroids. However, platelet count fell again when IFN- α 2A was resumed or when lymphoblastoid-IFN was administered. T-cell-mediated response and graft versus leukemia (GVL) effect may be brought on by overexpression of HLA molecules.¹² Recognition by CD8 cytotoxic/suppressor lymphocytes of peptides of tumoral or viral origin can lead to destruction of neoplastic or infected cells by the HLA class I restricted process.⁸ The same mechanism can also lead to platelet destruction. Therefore it is noteworthy that both the patients developed antiplatelet antibodies when a major karyotypic conversion was achieved. This could suggest that the antileukemic activity of IFN- α 2A may be mediated by an *autoimmune* GVL effect. On the other hand, we wish to stress the concept that the appearance of thrombocytopenia during IFN- α 2A-treated CML does not necessarily indicate a change in the biology of the disease, but could be due to the treatment itself, even through an autoimmune mechanism. Further study is needed to determine whether or not

differential stimulation or prolonged increase of HLA class I and class II antigen expression is of any significance for the immune recognition of malignant cells.

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