of p210 bcr/abl fusion protein in LG7 cells, detection and quantification of p210 bcr/abl transcript in these cells was done using a quantitative, competitive PCR.[9] The presence of bcr-abl transcripts in the LG7 cell line was further demonstrated by western blot analysis (data not shown). Our data suggest that ATRA can potentiate the inhibitory effects of IFNα both on Ph-negative and Ph+ leukemic cells. The mechanism (s) of synergism is unknown. It does not seem to be related to p210 expression, but appears to be influenced by preincubation of target cells with ATRA. These findings suggest that pretreatment with ATRA could induce activation of IFNα-induced genes[10] which in turn could favor the clinical response to IFNα.

Domenico Russo, Gianluca Tell,° Luciana Marin, Mario Tiribelli, Maria Alessandra Santucci, Carlo Pucillo°*

*Chair and Division of Hematology, Department of Medical and Morphologic Research; °Section of Immunology, Department of Biomedical Science and Technology, Udine University, Udine, *Institute of Hematology and Medical Oncology "L. e A. Seragnoli", Bologna University, Bologna, Italy*

Key words

ATRA, IFNα, Ph+ CML, p210 bcr/abl, cell lines

Acknowledgments

Work supported by Murst 40%,CNR contract #96. 00500.PF39 and by AIRC, Milan, Italy.

Correspondence

Dr. Domenico Russo, M.D., Clinica Ematologica, Policlinico Universitario, piazza S. Maria della Misericordia, Udine, Italy. Phone: international +39-0432-559662-64 – Fax: international +39-0432-559661– E-mail: domenico.russo@drmm.uniud.it

References

1. Sacchi S, Russo D, Avvisati G, et al. on behalf of the GER (Gruppo Ematologico Retinoidi). All-trans retinoic acid in hematological malignancies. An update. Haematologica 1997; 82:106-21.
2. Russo D, Regazzi M, Sacchi S, et al. All-trans retinoic acid (ATRA) in patients with chronic myeloid leukemia in the chronic phase. Leukemia 1998; 12:449-54.
3. Russo D, Marin L, Candoni A, et al. Pilot study of combined therapy with interferon-α, arabinosyl cytosine and all-trans retinoic acid in patients with chronic myeloid leukemia in the chronic phase. Haematologica 1999; 84:185-87.
4. Zheng A, Savolainen ER, Koistinen P. All-trans retinoic acid combined with interferon-α effectively inhibits granulocyte-macrophage colony formation in chronic myeloid leukemia. Leuk Res 1996; 20:243-8.
5. Mahon FX, Chahine H, Barbot C, Pigeonnier V, Reiffers J, Ripoche J. All-trans retinoic acid potentiates the inhibitory effects of interferon-α on chronic myeloid leukemia progenitors in vitro. Leukemia 1997; 11:667-73.
6. Greenberger JS, Sakakeeny MA, Humpries KC, Eaves CG, Ekner RJ. Demonstartion of a permanent factor-dependent multipotent (erythroid/neutrophil/basophil) hematopoietic progenitor cell line. Proc Natl Acad Sci USA 1983; 80:2931-5.
7. Laneuville P, Heisterkamp N, Groffen J. Expression of the chronic myelogenous leukemia-associated p210 bcr/abl oncoprotein in a murine IL-3 dependent myeloid cell line. Oncogene 1991; 6:275-82.
8. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immunol Methods 1983; 65:55-63.
9. Diviacco S, Norio P, Zentilin L, et al. A novel procedure for quantitative polymerase chain reaction by coamplification of competitive templates. Gene 1992; 122:313-20.
10. Matikainen S, Ronni T, Hurme M, Pine R, Julkunen I. Retinoic acid activates interferon regulatory factor-1 gene expression in myeloid cells. Blood 1996; 88:114-23.

Possible evolution of human parvovirus B19 infection into erythroleukemia

Sir,

Since its discovery in 1975, HPV B19 has been identified with an acute form of bone marrow failure in susceptible hosts. It is now well established that HPV B19 is cytotoxic for human erythroid precursors and causes a lytic process in infected cells.[1]

We report the case of a patient with HPV B19 bone marrow infection who developed erythroleukemia (EL).

A 69-year old man was admitted to a local hospital following several days of high fever and pancytopenia. The patient was given antibiotics and several units of red blood cells and then transferred to our institution for further evaluation.

Cytologic and histologic examination of bone marrow cellularity showed markedly reduced and dysplastic erythropoiesis. Blasts cell such as myeloblasts and monocytic blasts accounted for 20% of bone marrow cells. We were impressed by the high number of giant, frequently binucleated, erythroblasts, some of which were morphologically normal while others showed atypical irregularly-shaped nuclei, prominent nucleoli and vacuoles (Figure 1a). Cell pictures suggesting a fusion phenomenon were occasionally observed (Figure 1b). A provisional diagnosis of myelodysplastic syndrome was formulated.

One week after admission a rapid increase in WBC, platelet and reticulocyte count was observed. Bone marrow examination was repeated ten days after admission; the marrow showed marked erythropoietic hyperplasia, a few giant proerythroblasts were still present and there were numerous megakaryocytes while the number of blast cells was reduced. A clinical diagnosis of transient aplastic crisis due to HPV B19 was considered. Serologic examination revealed the presence of elevated anti-HPV B19 IgM antibodies and a low level of IgG, by ELISA immunoassay. Virus DNA, tested by PCR, was negative. In the following days a dramatic reticulocytosis followed, the blood count returned to normal values, the patient's clinical condition improved and he was discharged. He remained in good health for the next six months. He then started complaining of

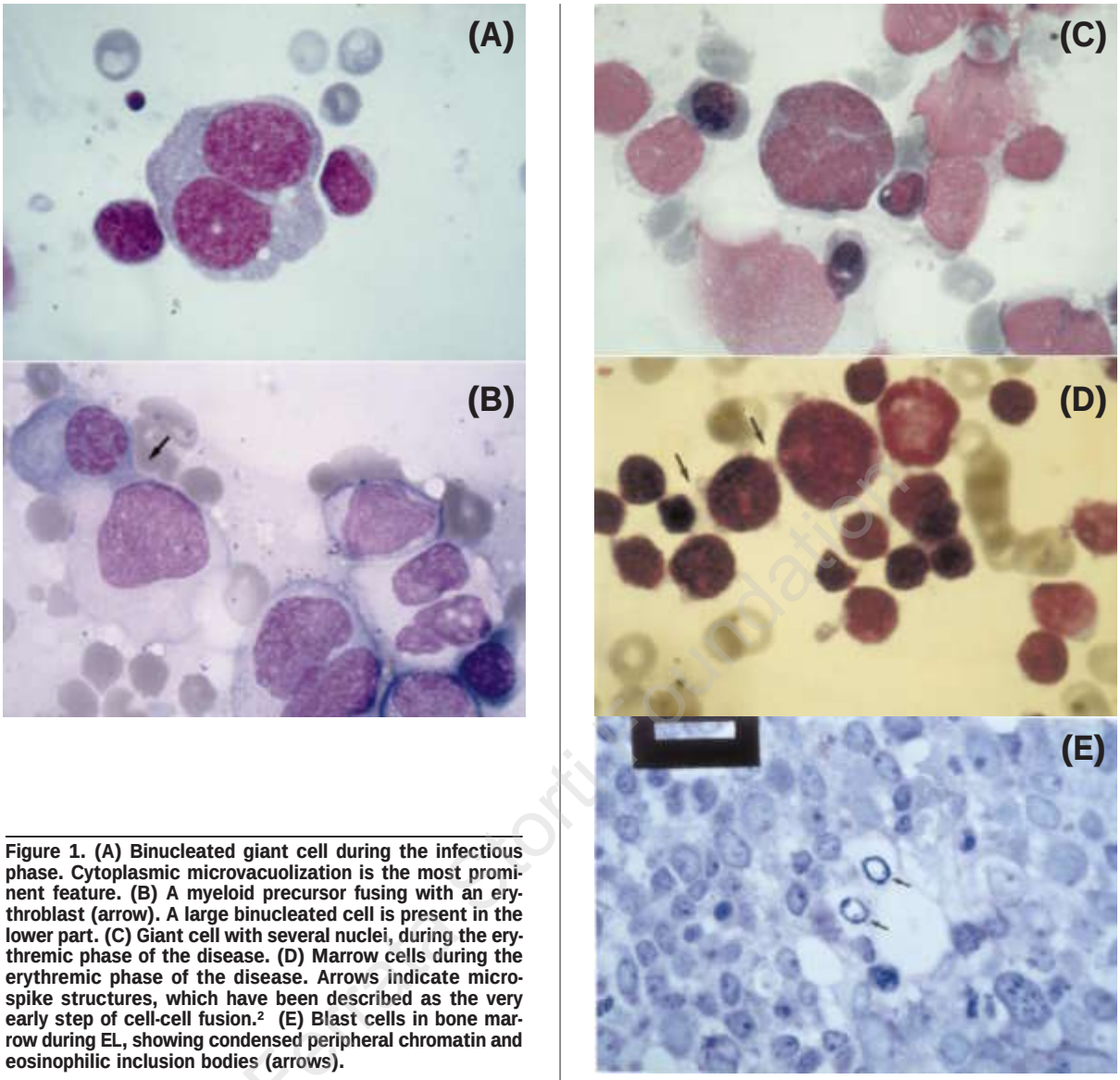

Figure 1. (A) Binucleated giant cell during the infectious phase. Cytoplasmic microvacuolization is the most prominent feature. (B) A myeloid precursor fusing with an erythroblast (arrow). A large binucleated cell is present in the lower part. (C) Giant cell with several nuclei, during the erythremic phase of the disease. (D) Marrow cells during the erythremic phase of the disease. Arrows indicate microspike structures, which have been described as the very early step of cell-cell fusion.[2] (E) Blast cells in bone marrow during EL, showing condensed peripheral chromatin and eosinophilic inclusion bodies (arrows).

fatigue and exertional dyspnea for which he was admitted to our hospital. His Hb level was 8.9 g/dL, WBC count was 2.1×10^9/L. A peripheral blood examination showed the presence of many blast cells (57%). Cytologic and histologic examination showed a hypercellular marrow with 90% agranular blasts. Residual cells comprised erythroblasts with a variety of abnormal cytologic features, from slight megaloblastic characteristics to vacuolization and the presence of bi- and multinucleated giant cells (Figure 1c). Again pictures suggestive of fusion phenomena could be observed, with morphologic details reminiscent of the characteristic features of cell fusion as described in HIV-induced cell-cell fusion: *cells made contact by using microspikes to touch and adhere to adjoining cells*[2] (Figure 1d). A preliminary diagnosis of acute leukemia was made and later confirmed by established cytochemical studies. Reactivity with anti-glycophorin A was strongly positive in 60% of blasts (APAAP stain with hematoxylin counterstain). A diagnosis of EL was made.

The appearance of EL in a patient who suffered an infectious disease of the erythron a few months before prompted us to investigate whether the leukemic cells where harboring HPV B19. B19 DNA in marrow cells was repeatedly absent when searched for by an *in situ* hybridization assay.[3] Despite courses of cytotoxic therapy and intensive supportive measures, the patient succumbed to an overwhelming infection.

Our findings suggest the evolution of an acute, primarily self-limiting HPV B19 transient aplastic crisis into EL. The characteristic giant proerythroblasts in the context of acute bone marrow failure first suggested the diagnosis of HPV B19 infection; additionally we observed multiple forms of bi-multinucleated giant cells and occasional morphologic evidence of cell fusion of erythroid precursors. The presence of

hybrid (myeloid-erythroid) blasts has been proposed to be a characteristic bone marrow feature in EL, but has not as yet been emphasized as a morphologic feature of the disease.[4] Erythroblasts with peripheral chromatin condensation are sufficiently characteristic for a provisional diagnosis of B 19 infection to be made on the histology[5] and rare erythroblasts with this morphologic feature could be observed (Figure 1e). While we can firmly correlate the infectious phase of the disease to HPV B19, the association with the neoplastic phase is only hypothetical because of the absence of the virus in the marrow cells when searched for by *in situ* hybridization.[6]

An alternative interpretation is that the patient had already subclinical EL which was unmasked by HPV B19 infection. If the virus hit compromised marrow, in which a shrunken compartment of normal hemopoiesis coexists with a still subclinical competing leukemia clone, pancytopenia may follow. Resolution of the virus infection might have given the normal marrow a chance to re-establish normal blood counts transiently, but EL finally exapanded and took over. Since this is a case report it would be inappropriate to draw general conclusions. Nevertheless interesting speculation arise from this report: is there an association between HPV B19 and EL and can cell fusion play a role in the cytogenesis of giant multinucleated erythroblasts?

Gianmaria Sitar, Carlo L. Balduini,* Luigi Manenti,* Alessandro Castello,° Dario Balanzin,* Edoardo Ascari**

**Institute of Internal Medicine and Oncology and °Institute of Pathology, University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy*

Key words

Parvovirus B19 infection, erythroleukemia, cell fusion.

Acknowledgments

We are indebted to Dr. Musiani, Division of Virology, University of Bologna, for performing in situ hybridization studies.

Correspondence

Gianmaria Sitar, MD, Istituto di Medicina Interna e Oncologia Medica, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy. Phone: international +39-0382-502567 – Fax: international + 39-0382-526223.

References

1. Brown KE, Young NS, Lin JM. Molecular, cellular and clinical aspects of parvovirus B19 infection. Crit Rev Oncol Hematol 1994; 16:1-31.
2. Dimitrov DS, Blumenthal R. Photoinactivation and kinetics of membrane fusion mediated by the human immunodeficiency virus type 1 envelope glycoprotein. J Virol 1994; 68:1956-61.
3. Gentilomi G, Zerbini M, Musiani M. In situ detection of B19 DNA in bone marrow of immunodeficient patients using a digoxigenin-labelled probe. Mol Cell Probes 1993; 7:19-24.
4. Atkinson J, Hrisinko MA, Weil SC. Erythroleukemia: a review of 15 cases meeting 1985 FAB criteria and survey of the literature. Blood Rev 1993; 6:204-14.
5. Kurtzman GJ, Ozawa K, Cohen BJ, Hanson G, Oseas R, Young NS. Chronic bone marrow failure due to persistent B19 parvovirus infection. N Engl J Med 1987; 317:287-94.
6. Heegaard ED, Myhre J, Hornsleth A, Gundestrup M, Boye H. Parvovirus B19 infections in patients with chronic anemia. Haematologica 1997; 82:402-5.

Pulmonary thromboembolism in thalassemia intermedia patients

Sir,

We[1] and others[2] have reported thromboembolic phenomena in patients with β-thalassemia major (TM). Thalassemia intermedia (TI) patients have a phenotypically milder disease, not requiring regular blood transfusions.[2] However, TI also predisposes to thrombotic events, similar to the life-long hypercoagulable state that exists in TM patients.[2,3] It is unknown whether TI patients who suffer thrombotic events have other inherited or acquired predisposing risk factors. We report the cases of two TI patients with no other risk factors for thrombosis who suffered from potentially life-threatening pulmonary thromboembolism. Both patients presented with increasing dyspnea of recent onset. Investigations revealed bilateral pulmonary thromboembolism. Pertinent clinical details are summarized in Table 1. The results of a screen for hypercoagulable states are shown in Table 2.

These two patients with TI suffered from bilateral pulmonary thromboembolic disease with unusual features. No risk factors (other than thalassemia) were present. Furthermore, no source of emboli could be

Table 1. Clinical details of the patients.

	Patient #1	*Patient #2*
Age (years)/Sex	51/Female	46/Female
Genotype	IVS1nt6/?	β039/αααα
Splenectomy	yes	yes
Cholecystectomy	yes	yes
Chelation therapy	no	irregularly
Blood transfusions	rare	rare
Hb (g/dL)	9.0	8.0
WBC (x10^9/L)(% normoblasts)	90.0 (90%)	22.9 (63%)
Platelets (x10^9/L)	650	580
PO_2 (mm Hg)	59	66
PCO_2 (mm Hg)	38	35
Ventilation/Perfusion scan		
Ventilation	normal	normal
Perfusion	4 segmental defects	bilateral lower lobe defects
Abdominal ultrasound	normal	normal
Other imaging studies	doppler of legs normal	venography normal