Increased proportion of CD10-positive cells in a boy affected with common acute lymphoblastic leukemia in consolidation therapy

Sir,

We describe the case of a 6-year-old boy affected with a common acute lymphoblastic leukemia (C-ALL) of L1 morphology. He started induction therapy according to the AIEOP 9102 protocol and achieved complete remission (CR) in January 1994. In September 1994, at the end of consolidation therapy, the patient underwent a routine bone marrow aspirate which revealed the presence of 47% undifferentiated cells resembling the morphology of L1 ALL. Peripheral leukocytes were 4x10^9/L with 60% neutrophils and 40% lymphocytes. Immunological characterization of these cells showed the same immunophenotype observed at the onset of the disease, i.e. TdT+, HLA-DR+, CD34+, CD19+, CD10+. Bone marrow cell genotypic analysis, performed with the Southern blot technique using ECO-RI, HIND III and BAM HI restriction DNA endonucleases for IgH genes and human placenta cells as control, revealed a monoclonal rearrangement of IgH chain genes at the onset of ALL (Figure 1, left), while it gave a germline configuration at the time of the suspected relapse (Figure 1, right). A few days later, just before the scheduled re-induction chemotherapy, the patient developed a de novo abdominal herpes zoster infection and thus specific therapy with i.v. acyclovir was administered, producing complete resolution of the infection. Antibodies to HIV, CMV, EBV and toxoplasma were negative. A re-evaluation bone marrow aspirate revealed a picture of CR, which continues at this writing with the patient off therapy and after a follow-up of 24 months from the infection.

Figure 1. Left: IgH gene configuration in M.S. at the onset of C-ALL. Samples 1 and 2 digested with BAM HI, 3 and 4 with ECO-RI, 5 and 6 with HIND III. Lane 1, 3, 5: human placenta cells. Lane 2, 4, 6: patient.
Right: IgH gene configuration at the suspected relapse. Samples 1 and 2 digested with BAM HI, 3 and 4 with ECO-RI, 5 and 6 with HIND III. Lane 1, 2, 3: human placenta cells. Lane 2, 4, 6: patient. Arrows indicate the monoclonal rearrangement bands.

An increase of bone marrow cells expressing the B-cell immature phenotype has been described in children affected with C-ALL, even several months after discontinuing chemotherapy, as well as in patients treated for AML with autologous bone marrow transplantation. Moreover, a high percentage of bone marrow cells expressing this phenotype has also been observed in children affected with non-malignant hematological disorders such as ITP or agranulocytosis. Finally, the CD10 antigen, normally expressed on B-lymphoid cells of fetal bone marrow and physiologically persisting in considerable amounts in the bone marrow of normal children, decreases with advancing age and almost entirely disappears in adult age. Thus, the B-cell immature phenotype can be found rather frequently in children’s bone marrow. On the other hand, although normal lymphoid cells are easily distinguishable from leukemic blast cells morphologically, sometimes the differential diagnosis can be very difficult. This is especially true when bone marrow cells resemble the so-called hematogones, which are TdT+, HLA-DR-, CD34-Helper, CD20+ stem cells. In these cases, only genotypic analysis can be conclusive in discriminating between normal cells and the neoplastic clone. Our case can be included in this category. Our patient’s bone marrow cells showed morphology suggestive of L1 type with a immunophenotype consistent with the C-ALL pattern. It is evident that this bone marrow picture was the consequence of the herpes zoster infection. To reinforce the benign nature of this process, a germline configuration of IgH chain genes was found in contrast to the monoclonal rearrangement detected at the onset of ALL. The patient is in continuous CR after 24 months of follow-up from the infectious episode and 29 months from the onset of ALL.

In conclusion, much caution in needed in some cases to avoid an incorrect diagnosis. In these patients careful morphological and immunophenotypic analysis, in addition to a genotypic study, should be performed before making the differential diagnosis.

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References

Thyroid function is not affected by second exposure to erwinia asparaginase for childhood acute lymphoblastic leukemia

Sir,

the use of L-asparaginase (ASP) has been associated with a transient reduction of serum thyroid hormones. In the AIEOP ALL-91 study, intermediate-risk children were randomized to receive 20 weekly high doses (HD: 25,000 IU/m²) vs 4 standard doses (SD: 10,000 IU/m² q 3 days) of Erwinia ASP (Erwinase, Speywood) intramuscularly as part of reinduction therapy, after having received 8 SD-ASP during induction therapy. This setting allowed us to explore the impact of different doses of ASP on thyroid function.

Blood samples were obtained from 23 consecutive patients (19 males, median age 6, range 2-13 years) randomized to SD (n=16) or HD (n=7) at weeks 0, 1, 4 (after completion of steroid therapy and 4 SD or 3 weekly HD of ASP), 9, 16, and 21 to 25 (during continuation therapy; the 7 patients in the HD-ASP arm had completed asparaginase therapy at week 20).

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Haematologica 1997; 82:507-512

letters to the editor
TRH stimulation test was performed in 11 patients (5 HD) at weeks 0 and 9. Plasma levels of thyroid hormones were determined within a single assay using commercially available kits, and differences between mean values were tested by the Student’s t-test for matched data. None of the patients developed hypothyroidism. Although thyroid hormone values remained within the reference range, all patients at week 1, after only one week of steroid therapy, showed a reduction in T3 and free-T3 (FT3) levels compared to basal values, with a contemporary transient increase of reverse T3 values. This difference was consistent in both the SD and HD groups, but was significant (p < .005) in the SD group only, due to the higher number of patients. Complete reversal of this pattern was evident at week 4 (Figure 1). FT3 remained low in all patients. TSH was not increased even at week 1 during the transient reduction of T3 and FT3. TBG, T4 and FT4 remained normal. TG was steadily low normal. The transient reduction of TBG was concomitant with that of thyroxine levels.

TRH stimulation test in 11 patients (5 HD) showed normal TSH peak time and height, and AUC at both evaluations. Thyroid function was not markedly impaired by second exposure to Erwinia ASP, either by short treatment at SD or by prolonged treatment at HD (cumulative dose 500,000 IU/m²). Both groups experienced a transient reduction of T3 and FT3, with an increase in rT3 but not TSH, that was evident after one week of steroid therapy before ASP was started. Thus a pathogenic role for ASP seems to be ruled out.

Ferster et al.3 reported a significant reduction of T3, T4, and TBG during induction, with a modest T3 and TBG decrease during reinduction, attributed to the higher dose of E. coli ASP (105,000 vs 40,000 IU/m²) and the more frequent administration (daily vs every 4 days) during induction. Despite a much higher dose of ASP in one arm, none of our patients showed marked thyroid dysfunction.

Failure to observe expected ASP-dependent biological effects might depend on ineffective asparagine depletion. Recently, greater clinical efficacy for the E. coli-ASP vs the Erwinia-ASP containing chemotherapy regimen,3 as well as lower asparagine depletion with Erwinia vs E. coli ASP have been reported.1 This is in keeping with the association of thyroid dysfunction and use of E. coli-ASP in Ferster’s study. In addition, failure to obtain asparagine depletion by second exposure to ASP may be due to antibody-mediated silent inactivation. Taken together, both of the above mechanisms might explain the failure to observe thyroid dysfunction as a consequence of second exposure to Erwinia ASP in our patients.

Autoimmune hemolytic anemia with anti-DC specificity following a primary infection by Varicella virus

Sir,

The association of hemolytic anemia and Varicella virus infection has been reported on three occasions.1–3 Cold antibodies of the IgM type were involved in two of them. Both of these showed anti-Pr specificity. We describe here an association of autoimmune hemolytic anemia, due to warm antibodies with anti-DC specificity linked to a primary infection by the varicella virus. In August 1993, a patient was admitted to the emergency room complaining of fever, myalgia and general malaise. The presence of a macula-vesicular eruption lead us to formulate a diagnosis of varicella. Physical examination revealed mild icterus and splenomegaly without adenopathies.

Routine laboratory analysis upon admission was as follows: hemoglobin 5.8 g/dL, HCT 16.4%, MCV 112 fl, leukocytes 5.7 × 10⁹/L (differential: 45% lymphocytes, 42% neutrophils), platelets 116 × 10⁹/L, ferritin 351 mg/dL, bilirubin 5.1 mg/dL (indirect 4.4), LDH 2047 U/L, haptoglobin was not detectable.

Morphological study determined the presence of 2% erythroblasts in the peripheral blood with no myelemia. The corrected amount of reticulocytes was 60%. The direct Coombs’ test was positive, with the presence of autoantibodies of the

Figure 1. Mean values of T3, FT3, rT3, T4, FT4 and TSH in 23 children affected by ALL, measures at week 0, 1, 4, 9, 16 and 21 to 25 of reinduction. Continuous line: patients treated with standard doses of ASP (n=16). Dashed line: patients treated with high doses of ASP (n=7). Shaded area: range of normal values.

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Unusual evolution to immunoblastic lymphoma of a case of Waldenström macroglobulinemia presenting with thrombocytopenia

Sir,

Waldenström macroglobulinemia (WM) is a lymphoproliferative disorder characterized by the secretion of elevated quantities of monoclonal IgM; approximately 6% of cases evolve to immunoblastic lymphoma (IL). On the other hand, the association of lymphoproliferative processes with thrombocytopenia is well known. We report a 65-year-old woman who came to our service with mucocutaneous bleeding and thrombocytopenia as the initial manifestations of WM which rapidly evolved to IL. At presentation the patient showed hepatosplenomegaly, platelets 14x10^9/L, leukocytes 6.9x10^9/L with normal differential count, and hemoglobin 10 g/dL. Immunoglobulin quantification was: IgG 1141 mg/dL, IgA 151 mg/dL, and IgM 333 mg/dL, with a normal immunoelectrophoretic pattern. Anti-platelets antibodies (IgG and IgM) were strongly positive. Marrow aspiration revealed abundant megakaryocytes and peripheral blood immunophenotype showed 30% CD19+, CD22+, Dr+, FMC7++ Iymphocytes, positive surface immunoglobulins, with lambda monoclonality (++), CDS+, CD25, and CD10-. Corticoid treatment at a dose of 2 mg/kg/day was begun and produced a progressive rise in the hemoglobin (14 gr/dL). At that time, the patient still showed slight hemolysis with thrombocytopenia. Following splenectomy there they fell to 10x10^9/L. Abdominal CT was normal, as were new anti-D antibodies (IgG and IgM). Variants of the D antigen were ruled out using a panel of anti-D monoclonal sera. The patient's genotype, determined by saline monochlonal sera, was CDe/CDe. Anti-DNA and ANA antibodies were negative. An immunohematological study carried out at the end of the disease evolution. To our knowledge, only one other such case has been reported previously.

References


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Letters to the Editor

Figure 1. Microscope examination of a cervical peripheral lymph node (H.E. 400x: immunoblastic lymphoma (polymorphic immunocytoma). Diffuse proliferation of lymphoplasmacytoid cells and large cells with numerous mitoses are shown.
A rapid and sensitive methods for the analysis of von Willebrand factor multimeric structure

Sir,

Human von Willebrand factor (vWF) mediates platelet adhesion and thrombus formation under high shear stress conditions; moreover, it binds and stabilizes procoagulant factor VIII in circulating blood.1 Quantitative and qualitative vWF abnormalities lead to a congenital bleeding disorder: von Willebrand disease (vWD). Mature vWF circulates in plasma as a series of multimers ranging in size from 450 kDa to in excess of 10,000 kDa.1 The multimeric structure of vWF can be demonstrated in normal subjects and in patients with different types of vWD, with the exception of the severe form of the disease.1 Multimeric analysis of plasma and platelet vWF is widely used as a diagnostic tool in screening and characterizing vWD; moreover, it is useful in the quality control of therapeutic plasma concentrates containing vWF.

We report a rapid, sensitive method for multimeric analysis of vWF. The procedure is based on vertical SDS-agarose mini-gel electrophoresis, followed by electroblotting and alkaline phosphatase immunostaining. Electrophoresis was performed on a vertical mini-gel apparatus (Mini-Protean II, Bio-Rad, Hercules, CA, USA) using the discontinuous buffer system of Ruggeri and Zimmerman.4 Separating gel consisted of 1.1% low-resolution or 2.6% high-resolution low gelling temperature agarose; stacking gel consisted of 0.8% high gelling temperature agarose. Citrated plasma was diluted 1:10 in sample buffer and incubated at 60°C for 15 min; 10 µL samples were applied to the wells. Electrophoresis was carried out at 60V for 20 min and then was continued for 6h at a constant 35V. Electrophoresis buffer was composed of 50 mM Tris, 384 mM glycine, 2 mM EDTA, 0.1% SDS, pH 8.35; the buffer temperature was held at 16°C. Gels were then transferred onto nitrocellulose filters by overnight blotting at 450 mA (50 mM phosphate buffer, 0.04% SDS, pH 7.4). After blocking non-specific sites, filters were incubated in anti-vWF primary antibody (Dakopatts, Glostrup, Denmark) at 4°C for 3h. The membranes were then washed and incubated in alkaline phosphatase-conjugated anti-rabbit IgG (ICN, Costa Mesa, CA, USA) at 4°C for 2h. Finally, vWF multimers were visualized by using BCIP/NBT as chromogenic substrate. The good transfer efficiency allowed us to obtain a normal pattern of more than 20 multimers by low resolution analysis (Figure 1), thus facilitating differential diagnosis between type 1 and most forms of type 2 vWD. High resolution analysis (Figure 1) separated the structure of the smallest vWF multimers into five bands. This gel was suitable for characterizing type 2 WD samples. A total working time of about 36h is needed to complete multimeric analysis; on the other hand, a minimum of 5 days is needed when multimer visualization is performed by autoradiography. Moreover, the disadvantages and hazards of handling radioactive materials are avoided. Compared to the horizontal method, the vertical electrophoretic system enabled us to optimize the buffer-gel contact and to prevent agarose drying. There is no fading of nitrocellulose filters developed by the alkaline phosphatase substrate; dried filters can be scanned by a densitometer and stored. Finally, the mini-gel system minimizes reagent consumption.

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CD5/CD19/CD23 chronic lymphocytosis

Sir,

the characterization of the chronic lymphoid leukemias is based on cell morphology and immunological markers.1 Difficulties in establishing defining criteria for each disease derive from the existence of overlapping features among the various disorders and a degree of variability in morphology, histology and phenotypic profile within the same entity. We report a case of B-cell lymphoproliferative disorder with a potentially confusing phenotype. A 48-year-old woman was admitted to our Hospital for anemic syndrome. Four years earlier she had been diagnosed as suffering from chronic lymphocytic leukemia (CLL) for an isolated peripheral blood lymphocytosis of 42 × 10^9/L. Immunophenotype performed at that time disclosed a positivity for CD5, CD19 and CD23. Bone marrow biopsy showed a diffuse infiltration by mature lymphocytes with 12% plasma cells. Marked splenomegaly with a monoclonal IgM paraprotein

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Figures 1. Immunoenzymatic staining of plasma vWF multimers by low resolution (LR) and high resolution (HR) analysis. 1. normal plasma; 2: type 1 vWD; 3: type 1 vWD after DDAVP infusion; 4: type 2A vWD; 5: type 2B vWD; type 3 vWD; 7: normal plasma. Arrowheads indicate the start of separating gel (cathode).
(48.55 g/L) was detected. She received treatment with three courses of chlorambucil and prednisone and responded well.

On current admission blood cell counts were as follows: hematocrit 22%, leukocytes 71.37 x 10^9/L (5% neutrophils, 92% lymphocytes with lymphoplasmacytic appearance), platelets 66 x 10^9/L. IgM paraprotein level was 60 g/L. Immunophenotype of the leukemic population gave the following results: CD5, CD19, CD23, CD22, CD25, cytoplasmic CD79a and FMC7 positive; CD10, CD103, CD38, BB-4 and CD11c negative. Smlg expression in the membrane was strong (Figure 1).

A diagnostic problem that often arises is the differentiation of CLL with lymphoplasmacytoid features from lymphoplasmacytic lymphoma (LL) and splenic lymphoma with villous lymphocytes (SLVL). In all three diseases a paraprotein IgM can be found. LL should always be considered in cases of CD5/CD19/CD23 chronic lymphoproliferative disorders.

Although rare, leukemic presentation can be misleading, especially if morphologic and clinical features are not so straightforward as in the case under discussion. Recently, immunophenotypic score systems have been proposed to differentiate between CLL and other chronic lymphoproliferative disorders. This distinction is clinically important since therapeutic implications are derived from a correct diagnosis.

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Leukemic meningitis in a patient with B-cell prolymphocytic leukemia

Sir,

B-prolymphocytic leukemia (B-PLL) has become recognized as a morphologic variant of B-chronic lymphocytic leukemia (B-CLL). Its prognosis and response to treatment are less favorable than those of CLL, with a median of survival of three years. Clinical syndromes related to involvement of the central nervous system by mature B-cell leukemias are rare. To the authors’ knowledge, only six cases of meningeal involvement have been reported in B-PLL. Here we describe a case with a poor outcome in spite of intensive systemic and intrathecal therapy.

A 75-year-old woman was admitted in our Hospital in June 1995 because of general fatigue and weight loss. Physical examination showed splenomegaly of 3 cm below the costal margin. Laboratory data revealed: leukocytes 84.9 x 10^9/L with 85% prolymphocytes, hemoglobin 10.3 g/dL, platelets 116 x 10^9/L. Serological tests for hepatitis C virus were positive. Immunophenotyping of peripheral blood demonstrated monoclonal B-lymphocytes expressing CD19 (95%), CD22 (95%), FMC7 (58%), CD5 (98%), CD10 (94%), CD23 (30%) and w light chain (strong). Bone marrow biopsy showed a diffuse infiltration by prolymphocytes. Thoracic and abdominal CT scans were normal. A diagnosis of B-PLL was made and treatment with chlorambucil and prednisone was given. Two weeks later the patient developed dizziness and diplopia. Neurologic examination showed left 6th nerve palsy without other motor deficiencies. An Ommaya reservoir was inserted in the patient and intraventricular treatment with weekly methotrexate, cytosine arabinoside and dexamethasone was started. At that time, systemic chemotherapy with the CHOP regimen was also initiated. After the first cycle of intrathecal chemotherapy the patient showed a marked improvement in the neurologic picture and she became asymptomatic after six cycles. She did well until two months later when right 7th nerve palsy and somnolence developed. Physical examination and hematological studies were similar to those at initial diagnosis. At that time, a cerebral CT scan again showed no abnormalities. CSF analysis revealed an elevated protein content (450 mg/dL) with normal glucose. There were 20/µ prolymphocytes and 300/µ red blood cells. Notwithstanding a new dose of intrathecal therapy, the patient’s neurologic status worsened and she died two weeks later, four months after initial diagnosis.

Symptomatic meningeal involvement is a rare complication in mature B-cell malignancies. In our patient, meningeal leukemia was confirmed by the presence of prolymphocytes in the CSF and by the response to intrathecal therapy. The literature experience suggests that effective control of meningeal disease in mature B-cell leukemias can be achieved with intrathecal chemotherapy. However, the patient described here achieved only a transient complete response and died with uncontrolled meningeal leukemia.
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