cent emission is subject to decay, plates should be evaluated as soon as possible. Our technique leads to the formation of a stable reaction colour: plates stored at 4°C up to four weeks retained initial staining characteristics;

4) unlike immunofluorescence, the procedure allows the evaluation of meg colonies in situ and to contemporarily observe positive, negative, and confluent colonies present in the visual field. Moreover, the technique allows the enumeration of colonies, visualization of intact colony morphology (Figure 1 A-C) and assessment of cell ploidy (Figure 1D). Finally, meg colonies immunoenzymatically identified can be stained further by May-Grünwald-Giemsa for a morphological examination of the single element9 and offers a permanent record.

Correspondence
Dr. Paolo Perutelli, Hematology Laboratory, Hematology and Oncology Dept., G. Gaslini Institute, largo G. Gaslini 5, 16147 Genoa, Italy.
Phone: international +39.10.5636277 • Fax: international +39.10.3776590.

References

Aggressive natural killer cell leukemia: report of a case in a Caucasian boy
ROGER MATEU, ISABEL BADELL, ANTONIO ALCALA,
CAMINO ESTIVILL, ENRIQUETA RUBIOL, JOSEP NOMDEDEU,
JESUS SOLER††
††Department of Hematology; †Department of Pediatrics, Hospital de la Santa Creu i Sant Pau, Barcelona; ‡Department of Hematology, Hospital General de Especialidades, Jaén, Spain
††Deceased

We report a case of aggressive natural killer cell leukemia (ANKL) in a Caucasian boy diagnosed on clinical, cytologic, immunophenotypic and genotypic grounds. An anomalous karyotype and integration of Epstein-Barr virus (EBV) within the leukemic population were identified.

Large granular lymphocytes (LGL) are a morphologically recognizable lymphoid subset comprising 10% to 15% of peripheral blood mononuclear cells. CD3+ LGL are natural killer (NK) cells that mediate nonmajor histocompatibility complex (MHC)-restricted cytotoxicity and do not express the CD3/T-cell receptor (TCR) complex or rearrange TCR genes. They show variable expression of the NK cell markers CD16, CD56 and CD57. CD3+ LGL are T cells that do express the CD3/TCR complex, often with expression of one or more NK cell markers, and rearrange TCR genes. These cells are cytotoxic T lymphocytes that can mediate nonMHC-restricted cytology.1

Generally, LGL leukemia is divided into two major categories based on the surface phenotype, T cell type and NK cell type.2 Aggressive NK cell leukemia (ANKL) was first described by Inamura et al. in 1990.3 These researchers reported their cases as aggressive NK cell leukemia/lymphoma but in the recent classification of NK cell and NK-like T-cell malignancies proposed by Jaffe,2 a distinction is made between ANKL cases and those considered as NK lymphomas (angioendothelial lymphomas), which usually present with localized extranodal disease (nasal and nasal-type NK cell lymphomas). Although there are sporadic reports of ANKL,3,9 mainly from Japan, little is known about the pathogenesis of this disease and no recurrent clonal chromosomal abnormality has been described to date.

A 16-year-old Caucasian boy was admitted to the hospital for malaise and jaundice. Physical examination and computerized axial tomography revealed marked hepatosplenomegaly but no lymphadenopathy.

Initial blood count showed a hemoglobin level of 11.9 g/dL, platelet count 19×10¹²/L and white blood cell count 12.69×10⁹/L with 18% circulating atypical lymphocytes. The biochemistry profile included serum γ-glutamyl transpeptidase (γ-GTP)
78 IU/L (normal, 8 to 38 IU/L), glutamic pyruvic transaminase (GPT) 140 IU/L (normal, 0 to 29 IU/L), glutamate oxaloacetate transaminase (GOT) 380 IU/L (normal, 0 to 52 IU/L), bilirubin 11.27 mg/dL (normal, 0 to 1 mg/dL), conjugated bilirubin 7.74 mg/dL (normal, 0 to 0.25 mg/dL), lactic dehydrogenase (LDH) 2540 IU/L (normal, 160 to 320 IU/L), triglycerides 330 mg/dL (normal, 70 to 150 mg/dL), proteins 5.79 g/dL (normal, 6.6 to 8.7 g/dL). Other laboratory data were within the normal range. Coagulation profile was: prothrombin time (PT) 55%, activated partial thromboplastin time (APTT) 53 seconds (27.9 s), fibrinogen 1.3 g/L (normal, 2 to 4 g/L), D-dimer 1500 ng/mL (normal, < 500 ng/mL). A bone marrow (BM) biopsy showed interstitial infiltration by atypical lymphocytes. A cavum biopsy and cerebrospinal fluid examination were normal. The surface phenotype of the peripheral blood (PB) and BM lymphocytes was CD1+, CD2+, CD3-, CD4-, CD5-, CD7+, CD8+, CD25+, CD38+, CD56+, CD94+ (BD), CD16+ (BD), HLA-DR+ (BD), CD38+ (BD), CD56+ (BD), CD94+ (HP-3B1 kindly provided by Dr. López-Botet, Hospital de la Princesa, Madrid), cytoplasmic CD3+ (BD), surface CD3- (BD), CD4- (BD), CD5- (BD), CD10- (BD), CD11b- (BD), CD13- (BD), CD14- (BD), CD19- (C), CD21- [Serotec Ltd, (ST) Oxford, England], CD22- (ST), CD25- (BD), CD33- (BD), CD34- (ST), glycoporphin A- [Immunotech, (I) Marseille, France], CD36- (I), CD41- (ST), CD61- (BD), CD57- (BD), αβ TCR- (ST), γδ TCR- (T cell diagnostics Inc, Cambridge, England), nuclear TdT- (Sera-Lab Ltd, Sussex, England). Cytogenetic study determined the karyotype to be 46, XY/46, XY, 6q-, -7, +mar, after short-term culture without stimulation and G-band-

![Figure 1. Peripheral blood smear (May-Grunwald Giemsa, x1000) showing atypical lymphoid cells with basophilic cytoplasm and azurophilic granules.](image)

![Figure 2. Southern Blot analysis of Epstein-Barr virus (EBV) termini. Identification of one band of 8.7 kb on BamH I digesta with a probe to the terminal repeat region of EBV shows the case (lane 1) contains a significant amount of homogeneous episomal EBV DNA indicative of a clonal population. Lane 7 represents a single 7.4 kb band detected in a non-Hodgkin lymphoma (NHL) from a HIV-positive patient. All other lanes corresponded to other NHL; none of these hybridized with the EBV probe.](image)
ing. The TCR-β gene was found to be in the germ-line configuration after analysis using a Cβ on BamHI and EcoRI digests. Southern blot analysis of EBV termini showed the presence of one 8.7 kb band on BamHI digests with a probe specific for the EBV genomic termini (Figure 2). A diagnosis of ANKL was formulated, but the patient died shortly afterwards due to multiorgan failure, 5 months after diagnosis.

A diagnosis of ANKL in our patient is supported on clinical, morphologic, immunophenotypic and genotypic grounds. Clinically, the entity is characterized by hepatosplenomegaly with disseminated disease, BM and PB involvement and an aggressive clinical course.2,3

In our case, atypical lymphocytes were CD2+ and expressed the NK markers CD56, CD16 and CD94. They did not express surface CD3, CD5, αβ TCR, or γδ TCR and presented TCR in the germline configuration. These findings argue against a T-cell lineage and strongly support NK lineage. On the other hand, CD8 and cytoplasmic CD3 expression (Leu4) have been reported more frequently in T-cell malignancies. Nevertheless, expression of both antigens has been described in cases considered to be of NK lineage.5,13

In fact, a subset of normal NK cells expresses CD8α,4 and although adult NK cells only express cytoplasmic CD3e, fetal NK cells express cytoplasmic CD3e, CD3α and CD3δ,14 a complex that is recognized by the leu4 antibody. On the basis of leu4 positivity and TCR germline configuration, some authors have hypothesized that the cellular origin of the maligniant population might correspond to these fetal NK cells.13 However, since there is little information available regarding the use of flow cytometry for the study of cytoplasmic CD3 in NK cell malignancies, we could not ascertain the true origin of these cells.

The clonal nature of the NK cell proliferation was demonstrated by the finding of a single band in Southern blot analysis using the EBV genomic termini probe, and by the detection of an aberrant karyotype. Although the association of EBV and ANKL is variable,2 the demonstration of clonal EBV episomal DNA in the neoplastic population raises the possibility of an etiological role for EBV in the leukemic transformation, as has been suggested by some authors.5 In contrast with the finding of an anomalous karyotype and clonal integration of EBV in our case and in other aggressive cases, mainly in Japanese patients,4 some studies in European and American patients with chronic CD3− LGL lymphocytosis were unable to demonstrate clonality or the presence of the EBV genome.4 Nevertheless, as noted above, progression from a chronic form to an aggressive phase has been previously reported. Further studies are thus needed to determine in which way EBV could contribute to the neoplastic transformation and to the aggressive course of the disease.

**Acknowledgments**

The authors wish to thank Dr López-Botet (Hospital de la Princesa, Madrid) for providing the HP-3B1 (CD94) monoclonal antibody.

**Correspondence**

J. Nomdedéu, Departament d’Hematologia, Hospital de la Santa Creu i Sant Pau, Avenida Sant Antoni Maria Claret 167, 08025 Barcelona, Spain.
Phone: international +34-3-2919290 • Fax: international +34-3-2919192.

**References**