Adenocarcinoma of the anal glands

1011

natural killer cell large granular lymphocyte type with HLA-DR-CD16-CD56bright+ phenotype

J Prieto, E Rios, A Parrado, A Martin, J M de Blas, J M Rodriguez

Abstract
The case is reported of a 45 year old woman with the rare leukaemia of natural killer cell large granular lymphocyte (NK/LGL) type. Cytometric analysis of leukaemic blasts showed that they were positive for CD2, CD38, and CD56 antigens but negative for a series of antigens including CD3, CD7, CD16, and HLA-DR. Rearrangements of the β T cell receptor, and heavy and κ immunoglobulin genes were not detected and neither were chromosomal abnormalities. Leukaemic blasts developed NK cytotoxicity. The patient failed to respond to aggressive chemotherapy and died three months after diagnosis. The lack of expression of HLA-DR is an extraordinary characteristic of this case, as all cases of acute NK cell leukaemias described to date expressed HLA-DR. The immunophenotype observed in the NK cell leukaemic blasts may represent the counterpart of a hypothetical normal cell precursor in an early stage of ontogenic NK cell development.

Keywords: natural killer, acute leukaemia, immunophenotype, development.

Natural killer (NK) cell proliferative disorders are very infrequent diseases that can be classified into chronic and acute forms. Acute NK cell leukaemia, referred to as NK/LGL leukaemia, has been described mainly in Japanese patients, although some European cases have been reported. NK cells express the CD3+CD56+ or CD3+CD16+ phenotypes, or both, and normally exist in the bone marrow, peripheral blood, and spleen. NK cells develop cytolytic activity against cells infected with viruses and also against certain tumour targets in a way not restricted to the expression of molecules of the major histocompatibility complex (MHC) on the target cell. All the cases of NK/LGL leukaemia so far described present the CD3+CD56+HLA-DR- phenotype. In this paper we present a case of NK/LGL leukaemia with CD3+CD16-CD56bright+HLA-DR- phenotype.

Case report
A 45 year old woman was admitted with a high fever (but without signs of infection), cephalgia and myalgia, and moderate splenomegaly. Her haematometric results were: haemoglobin 75 g/l, leucocyte count of 2.3 × 10^9/l, with a differential count of 57% segmented neutrophils, 5% monocytes, 26% lymphocytes, and 12% blasts with LGL morphology, and a platelet count of 90 × 10^9/l. Two days later her
pancytopenia had progressed: haemoglobin 60 g/l, leucocyte count of 1.2 x 10⁹/l, and a platelet count of 30 x 10⁹/l. Twenty days before entering hospital her laboratory analytical results were normal. The bone marrow aspirate was hypocellular with a 61% infiltration of blasts. The blasts presented a pleomorphic appearance with a very irregular nuclear and cellular outline, a moderately loose chromatin, and an abundant basophilic cytoplasm with small to medium sized azurophilic granules with a mainly centrosomic localization. Few cells had a visible nucleus. Ninety five percent of the blasts were acid phosphatase positive with a centrosomic granular pattern, and 7% were positive for periodic acid Schiff in the form of isolated granules. The blasts were negative for myeloperoxidase, Sudan black B, α-naphthyl acetate esterase, and chloroacetate esterase. Electron microscopic examination showed that the nuclei of the blasts were irregular in outline, had heterochromatin accumulations and frequently nucleoli, and that the cytoplasm had a prominent granulation with isolated bundles of microfilibrils. No karyotypic abnormalities were detected using chromosomal banding techniques. Southern blot analysis, using biotin labelled Jµ, Jδ, Jγ, Jε, and Jκ probes (Oncor, Gaithersburg, Maryland, USA) revealed that the β T cell receptor (TCR), and heavy and κ immunoglobulin (ig) genes were in germline configuration. These findings, together with immunophenotypic studies and analyses of cytolytic activity of the blasts, justified a diagnosis of NK/LGL leukaemia. Because of the poor response of the patient to the treatment followed in cases described in published reports, she was considered to be at high risk. Treatment with daunorubicin, prednisone, cyclophosphamide, vincristine, and L-asparaginase was initiated but complete remission was not achieved. The patient then received dexamethasone, vincristine, L-asparaginase, and high doses of cytosine arabinoside and methotrexate. She died of a pulmonary infection three months after diagnosis, in a phase of pancytopenia. Three days before death the bone marrow aspirate was hypocellular with a 97% blast infiltration.

IMMUNOPHENOTYPIC ANALYSIS
Surface marker analysis was done by double immunofluorescence flow cytometry. The following fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies were used: anti-CD1 (OKT-6), CD2 (Leu-5b), CD3 (Leu-4), CD4 (Leu-3), CD5 (Leu-1), CD7 (Leu-9), CD8 (Leu-2), CD11b (Leu-15), CD11c (Leu-M5), CD13 (Leu-M7), CD14 (Leu-M3), CD15 (Leu-M1), CD16 (Leu-11), CD19 (Leu-12), CD20 (Leu-16), CD22 (Leu-14), CD33 (Leu-M9), CD36 (OKM-5), CD38 (Leu-17), CD42b (DAKO-CD42b), CD56 (Leu-19), CD57 (Leu-7), CD61 (Dako-IIIa), CD71 (Dako-CD71), and myeloperoxidase (Dako-MPO).

The OK series was manufactured by Ortho Diagnostics, Raritan, New Jersey, USA, the Leu series by Becton Dickinson, San Jose, California, USA, and Dako series by Dako, Glostrup, Denmark. The panel of monoclonal antibodies studied also included: anti-CD10 (CALLA), CD25, CD34 (HPCA-2), HLA-DR, TCRβ, and TCRγδ from Becton Dickinson; c-kit, TdT, and glycoporphin-A from Immunotech, Marseille, France; and polyclonal anti-κ and anti-λ antibodies from Dako.

Bone marrow leukaemic blasts were isolated by Ficoll density gradient centrifugation and suspended to a concentration of 4 x 10⁸/ml in phosphate buffered saline (PBS) with 10 mg/ml bovine serum albumin. A volume of 100 ml cell suspension was incubated with 20 mg of each monoclonal antibody for 30 minutes at 4°C in the dark. Cells were washed and suspended in 100 ml PBS containing 1 mg/ml sodium azide. Percentages of fluorescent labelled cells were determined from 10 000 acquired cells on a FACScan flow cytometer (Becton Dickinson).

CYTOTOXICITY ASSAY
The human erythroid K562 and lymphoid Raji cell lines were used as targets to measure NK and spontaneous LAK activity, respectively, as described previously. Briefly, the patient’s mononuclear cells, obtained by Ficoll density gradient centrifugation, and 5 x 10⁷ target cells were labelled by incubation with 100 mCi ⁵¹Cr for 90 minutes. In triplicate, varying numbers of patient’s leukaemic cells and 5 x 10⁷ target cells were co-cultured at ratios of 40:1 and 80:1 in RPMI-1640 medium containing 10% fetal calf serum in 96-well round bottomed microtitre plates for four hours. Control wells containing target cells alone in the medium or 2% sodium dodecyl sulphate (SDS) were used to measure spontaneous release (SR) and maximum release (MR) of chromium, respectively. The supernatants were collected using the Skatron supernatant collection system and measured in a gamma counter. The percentage of experimental lysis was calculated according to the formula: % lysis = [100 × (ER – SR)/(MR – SR)], where ER represents experimental release of ⁵¹Cr.

Results
Cytometric analysis of the leukaemic blasts revealed that they were positive for the CD2 (96%), CD38 (83%) and CD56 (96%) antigens, but negative (0%) for the other markers studied. The negativity for the HLA-DR marker was confirmed using the alkaline phosphatase anti-alkaline-phosphatase (APAAP) technique.

The blasts developed high levels of NK activity (36% and 52% lysis on K562, at ratios of 40:1 and 80:1, respectively) and of spontaneous LAK activity (59% and 68% lysis on Raji, respectively).

Discussion
We describe a case of the rare NK/LGL leukaemia with a CD3⁺CD16⁺CD56⁺ phenotype. According to our data, approximately 5% of the NK cells in the peripheral blood of normal individuals present the same phenotype. Although the ontogenic
Presence of the bcr/abl rearrangement in a patient with chronic neutrophilic leukaemia

C Christopoulos, K Kottoritis, V Mikraki, E Anevlavis

Abstract
An 83 year old woman presented with a myeloproliferative disorder involving the myeloid and megakaryocytic lines, and characterised by mature neutrophil leucocytosis. There was a high/normal neutrophil alkaline phosphatase activity and absence of the Philadelphia chromosome, features compatible with a diagnosis of chronic neutrophilic leukaemia (CNL).

Southern blot analysis of the patient's DNA revealed the presence of the bcr/abl rearrangement. Combined with a previous report of detection of Ph1 chromo-

Leukaemia of natural killer cell large granular lymphocyte type with HLA-DR-CD16-CD56bright+ phenotype.

J Prieto, E Ríos, A Parrado, A Martín, J M de Blas and J M Rodríguez

doi: 10.1136/jcp.49.12.1011