carcinoma. The differential diagnoses include squamous cell carcinoma of the anal canal with mucinous histiocytes, apocrine carcinoma and Paget's disease of the perianal skin. An adenocarcinoma of the rectum may extend into the anal canal but is likely to be associated with an obvious intraluminal tumour. The association of carcinoma with fistula-in-ano is well known: this occurs when a chronic fissure or fistula has been present for 20 years or more. The carcinoma which develops is often colloid in type, with considerable mucin production.² These cases have to be distinguished from invasive carcinomas presenting as non-healing fistulae³ which have a much shorter history.

Reported cases of anal gland carcinoma have attracted controversy previously because of the difficulty in demonstrating a site of origin.^{2,3} The diagnosis can only be made with certainty

when the anal glands show in situ neoplastic change, as in the two cases presented here.

Our thanks are due to our surgical colleagues, Mr D J Galloway and Professor W D George, for permission to publish their cases.

- 1 Wellman RF. Adenocarcinoma of anal duct origin. *Can J Surg* 1962;5:311-18.
- 2 Winkelman J, Grosfeld J, Bigelow B. Colloid carcinoma of anal gland origin. Report of a case and review of the literature. *Am J Clin Pathol* 1964;45:395-401.
- 3 Tarazi R, Nelson RL. Anal adenocarcinoma: a comprehensive review. *Semin Surg Oncol* 1994;10:235-40.
- 4 Abel ME, Chiu YSY, Russell TR, Volpe PA. Adenocarcinoma of the anal glands: Results of a survey. *Dis Colon Rectum* 1993;36:383-7.
- 5 Fenger C, Filipe MI. Mucin histochemistry of the anal canal epithelium. Studies of normal anal mucosa and mucosa adjacent to carcinoma. *Histochem J* 1981;13:921-30.
- 6 Grinvalsky HT, Helwig EB. Carcinoma of the anal function. Histologic considerations. *Cancer* 1956;9:480-8.
- 7 Rosser C. The relation of fistula in ano to cancer of the anal canal. *Trans Am Proct Soc* 1934;35:65-71.

J Clin Pathol 1996;49:1011-1013

Leukaemia of natural killer cell large granular lymphocyte type with HLA-DR⁻CD16⁻CD56^{bright+} phenotype

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

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.

(*J Clin Pathol* 1996;49:1011-1013)

Natural killer (NK) cell proliferative disorders are very infrequent diseases that can be classified into chronic¹ and acute² 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.³ All the cases of NK/LGL leukaemia so far described present the CD3⁻CD16⁺CD56⁺HLA-DR⁺ phenotype.^{2,4} In this paper we present a case of NK/LGL leukaemia with CD3⁻CD16⁻CD56^{bright+}HLA-DR⁻ phenotype.

Case report

A 45 year old woman was admitted with a high fever (but without signs of infection), cephalaea 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

Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen del Rocío, Servicio Andaluz de Salud, Sevilla, Spain

Correspondence to: Dr Prieto, Servicio de Hematología y Hemoterapia, Hospital Universitario Virgen del Rocío, Avda Manuel Siurot s/n, 41013 Sevilla, Spain.

Accepted for publication 20 July 1995

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

pancytopenia had progressed: haemoglobin 60 g/l, leucocyte count of $1.2 \times 10^9/l$, and a platelet count of $30 \times 10^9/l$. 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 localisation. Few cells had a visible nucleolus. 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 microfibrils. No karyotypic abnormalities were detected using chromosomal banding techniques. Southern blot analysis, using biotin labelled $J_{\beta I}$ - $J_{\beta II}$, J_H , and J_K 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, TCR $\alpha\beta$, and TCR $\gamma\delta$ from Becton Dickinson; c-kit, TdT, and glycophorin-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 $4 \times 10^6/ml$ 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.⁵ Briefly, the patient's mononuclear cells, obtained by Ficoll density gradient centrifugation, and 5×10^6 target cells were labelled by incubation with 100 mCi ^{51}Cr for 90 minutes. In triplicate, varying numbers of patient's leukaemic cells and 5×10^5 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 = $100 \times (ER - SR)/(MR - SR)$, where ER represents experimental release of ^{51}Cr .

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^{bright+} immunophenotype. According to our data, approximately 5% of the NK cells in the peripheral blood of normal individuals present the same phenotype. Although the ontogenic

development of the NK cells has not been elucidated, it has been suggested that these cells could be precursors of those with the CD3⁺CD16⁺CD56^{dim} phenotype, which are the main NK cells in normal individuals. Increased counts of NK CD3⁺CD16⁺CD56^{bright} cells have been reported in a group of patients after bone marrow transplant or treatment with interleukin 2,⁶⁻⁸ suggesting that these procedures could mobilise these hypothetical precursor cells towards the circulating blood.

The phenotypes of tumour cells have often permitted the prediction of unknown links in T and B lymphoid differentiation. We believe that the phenotype in NK cell leukaemias could also provide clues for elucidating the ontogeny of NK cells. One antigenic characteristic common to all the NK/LGL leukaemias described in published reports, including our patient, is the lack of expression of CD7. This is a surface antigen expressed by normal T and NK cells. Some models postulate that in the differentiation of the NK cells, the expression of CD7 might be a very early occurrence,^{9,10} as is the case in T cell differentiation. Less than 2% of the NK cells in the peripheral blood of normal subjects have the CD7⁺CD56⁺ phenotype, supporting the probability that these are early precursors of CD7⁺CD56⁺ NK cells. However, the absence of CD7 in the leukaemic NK cells might indicate that this marker is expressed in NK cells following the expression of other markers, such as CD2 or CD56, which are expressed in all the cases of NK/LGL leukaemia reported to date.

HLA-DR is not expressed by the majority of normal NK cells, only by activated ones, so it should not be surprising that this feature is absent in our patient. The curious fact is that our patient is the only one who did not express HLA-DR among all the cases of NK/LGL leukaemias studied. In patients treated with interleukin 2, a small but significant (2–3% of peripheral blood lymphocytes) proportion of

cells with the CD3⁺CD7⁺CD8⁺CD16⁺CD56⁺⁺ HLA-DR⁺ phenotype can be measured,⁸ showing that this cell type exists in the peripheral blood, at least when given certain stimuli. The neoplastic cells observed in our patient could represent a leukaemic counterpart of this hypothetical NK cell precursor.

The present case had clinical and haematological features similar to typical Japanese NK/LGL leukaemia, which suggests it is the same entity presenting in a European patient. We conclude that the rare occurrence of NK/LGL leukaemia and the peculiar immunophenotype of the blast cells, which as far as we know is the first to be described, lends special interest to the present case.

- 1 Tefferi A, Li CY, Witzig TE, Dhodapkar MV, Okuno SH, Phylipy RL. Chronic natural killer cell lymphocytosis: a descriptive clinical study. *Blood* 1994;84:2721–5.
- 2 Imamura N, Kusunoki Y, Kawa-Ha K, Yumura K, Hara J, Oda K, *et al.* Aggressive natural killer cell leukaemia/lymphoma: report of four cases and review of the literature. Possible existence of a new clinical entity originating from the third lineage of lymphoid cells. *Br J Haematol* 1990;75:49–59.
- 3 Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. *Blood* 1990;76:2421–38.
- 4 Soler J, Bordes R, Ortuño F, Montagud M, Martorell J, Pons C, *et al.* Aggressive natural killer cell leukaemia/lymphoma in two patients with lethal midline granuloma. *Br J Haematol* 1994;86:659–62.
- 5 Parrado A, Rodriguez-Fernández JM, Casares S, Noguero P, Plaza E, Parody R, *et al.* Generation of LAK cells in vitro in patients with acute leukemia. *Leukemia* 1993;7:1344–8.
- 6 Jacobs R, Stoll M, Stratmann G, Leo R, Link H, Schmidt RE. CD16⁺CD56⁺ natural killer cells after bone marrow transplantation. *Blood* 1992;79:3239–44.
- 7 Weil-Hillman G, Fisch P, Prieve AF, Sosman JA, Hank JA, Sondel PM. Lymphokine-activated killer activity induced by in vivo interleukin 2 therapy: predominant role for lymphocytes with increased expression of CD2 and Leu19 antigens but negative expression of CD16 antigens. *Cancer Res* 1989;49:3680–8.
- 8 Parrado A. Análisis de la actividad citolítica natural y las subpoblaciones linfocitarias en las leucemias agudas: efecto in vitro e in vivo de la interleucina-2. PhD thesis. Seville: University of Seville, 1994.
- 9 Sánchez MJ, Spits H, Lanier LL, Phillips JH. Human natural killer cell committed thymocytes and their relation to the T cell lineage. *J Exp Med* 1993;178:1857–66.
- 10 Miller JS, Alley KA, McGlave P. Differentiation of natural killer (NK) cells from human primitive marrow progenitors in a stroma-based long-term culture system: identification of a CD34⁺ NK progenitor. *Blood* 1994;83:2594–601.

J Clin Pathol 1996;49:1013–1015

First Department of Internal Medicine, Agia Olga Hospital, Athens, Greece

C Christopoulos
K Kottoris
E Anevlavis

Choremeion Oncology Research Laboratory, Medical School, Athens University, Athens, Greece
V Mikraki

Correspondence to: Dr C Christopoulos, Vas. Alexandrou 7, Kifissia, Athens, 145 61 Greece.

Accepted for publication 4 September 1996

Presence of the bcr/abl rearrangement in a patient with chronic neutrophilic leukaemia

C Christopoulos, K Kottoris, 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-