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Blastic CD4 NK cell leukemia/lymphoma: a distinct clinical entity

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Abstract

We report the findings of three new cases of a distinct clinicopathologic natural killer (NK) cell malignancy characterized by cutaneous, nodal and bone marrow infiltration by $CD3^{-}CD4^{+}CD56^{+}$ NK blastic cells. Tumor cells were detected in bone marrow and in peripheral blood smears and showed finely distributed nuclear chromatin with nucleoli and a moderate amount of cytoplasm. Epstein-Barr virus (EBV) DNA was negative in the two tested cases. The immunophenotypes determined by flow cytometry were identical concerning $mCD3^{-}cytCD3^{-}CD4^{+weak}CD56^{+}$ HLA-DR^{+}. The TCR was in germline configuration in the two cases tested. NK cell activity was demonstrated only in one out of the two cases tested. The negative reactions with α-naphthyl-acetate-esterase (ANAE), CD11b and CD14 strongly suggested that the tumor cells were not of the monocytic lineage.

Keywords: $CD4^{+}CD56^{+}$; Blastic NK lymphoma; Epstein-Barr virus; NK cells

1. Introduction

The malignant natural killer (NK) cell lymphoproliferative disorders are characterized by the clonal proliferation of true NK cells ($CD3^{-}CD16^{\pm}CD56^{\pm}CD57^{\pm}$) or NK-like T cells ($CD3^{+}CD16^{\pm}CD56^{\pm}CD57^{\pm}$) and include aggressive NK cell leukemia, extranodal NK/T cell lymphoma, nasal type, and blastic NK cell lymphoma/leukemia [1–3]. Approximately 30 cases of blastic NK cell lymphoma/leukemia were described which were characterized by cutaneous, nodal and bone marrow infiltration with $CD3^{-}$, $CD4^{+}$ and $CD56^{+}$ NK blastic cells [2,4–15]. Most of these cases were associated with an aggressive clinical course. We report the findings of three new cases of this disease in Brazilian patients which were diagnosed in the leukemic phase.

2. Case reports

The clinical and hematological characteristics of the three patients are detailed in Table 1. All cases sought care because of cutaneous lesions. All presented in the leukemic phase with infiltration of peripheral blood and bone marrow. Tumor cells was detected in cerebrospinal fluid of case 2. All patients were HIV and HTLV-I negative.

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3. Material and methods

Lymphoid cells from peripheral blood, bone marrow, cerebrospinal fluid or from a lymph node cell suspension were stained with a panel of monoclonal antibodies by indirect single color or direct double or triple color immunofluorescence. Cell suspensions from lymph nodes was prepared by teasing out the lymph node immediately after the surgical biopsy. All antibodies were purchased from Becton Dickinson, San José, CA, except for CD122 (p75) which was purchased from Janssen Biochimica, Beerse, Belgium, and for TdT which was from Dako Coorporation, Glostrup, Denmark. All samples were analyzed with a FACScan (Becton Dickinson, Immunocytometry Systems, San Jose, CA) using the Cell Quest and the Paint a Gate softwares. The lymphocyte gate was performed on the basis of forward scatter (FSC) and side scatter (SSC).

3.1. NK activity

NK activity from peripheral blood tumor cells was tested against a ^{51}Cr-labeled K562 cell line at effector: target ratios of 160:1, 40:1, 10:1 and 2.5:1. as previously reported [16]. Briefly, after 4 h at 37 °C in a 5% CO_2 atmosphere, the cells were centrifuged, one-half of the supernatant was removed, and gamma radiation was counted. Specific cytotoxicity was calculated as (observed ^{51}Cr release − spontaneous ^{51}Cr release in medium)/maximum ^{51}Cr release in 2% Triton X.

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Table 1
Clinical and hematological characteristics of the patients

Patient	Age (year)/ sex	Duration of disease (months)	Cutaneous lesions	Lymph node	Liver (cm)	Spleen (cm)	Hb (g/dl)	WBC ($\times 10^9$/l)	Blast cell			Platelets ($\times 10^9$/l)	Clinical course
									Morphology	PB	BM		
1	18/M	4	Subcutaneous nodules, widespread purpuric plaques	Cervical, axillary, inguinal	0	4	14	6.2	LAL[a]	46	95	307	CHOP dead 60 days
2	68/M	18	Purpuric plaques on face and thorax	Supraclavicular	2	2	8.3	3.0	LAL[a]	57	100	71	CHOP—Bleo, M-BACOD dead 90 days
3	8/M	2	Subcutaneous nodules on right elbow and wrist	Cervical, axillary	0	0	9.6	5.6	LAL[a]	85	100	114	Treated as ALL[b] Complete remission for 7 years

[a] LAL, large agranular lymphoid cells; L.G.L., large granular lymphoid cells.
[b] Brazilian protocol for ALL GTBLI-93.

Table 2
Phenotypic characteristics, NK activity of peripheral blood cells, TCR configuration and Epstein-Barr virus

Patient		Phenotypic characteristics	NK activity	TCR	Epstein-Barr virus
1	Peripheral blood	$CD2^-$ $CD3^-$ $CD5^-$ $CD7^+$ $CD4^+$ $CD56^+$	Absent	Germline	Not done
	Bone marrow	$CD2^-$ $CD3^-$ $CD5^-$ $CD7^+$ $CD4^+$ $CD56^+$			
2	Peripheral blood	$CD2^+$ $CD3^-$ $CD5^+$ $CD7^+$ $CD4^+$ $CD56^+$	Present	Germline	Negative
	Bone marrow	$CD2^+$ $CD3^-$ $CD5^-$ $CD7^+$ $CD4^+$ $CD56^+$			
	Cerebrospinal fluid	$CD2^+$ $CD3^-$ $CD5^-$ $CD7^+$ $CD4^+$ $CD56^+$			
3	Bone marrow	$CD2^+$ $CD3^-$ $CD5^-$ $CD7^+$ $CD4^+$ $CD56^+$	Not done	Not done	Negative

3.2. Immunogenotyping

TCR rearrangements were investigated using DNA from fresh tumor cells. For patients 1 and 2, junctional sequences of the *TCRγ* and *TCRδ* genes were investigated by the polymerase chain reaction (PCR) as previously reported [17].

3.3. Detection of EBV genome

Epstein-Barr virus (EBV) DNA from frozen tumor cells was tested by PCR as previously reported [17]. Total cellular DNA was extracted from 200 μl of frozen peripheral blood mononuclear cells using a QIAamp Blood Kit (QIAgen Ind., Chatsworth, CA, USA) according to manufacturer instructions. The PCR primers for this assay were selected from the *Bam*H1W repeat region of the EBV genome. The upstream and downstream primer sequences were 5′-CTTTAGAGGCGAATGGGCGCCA-3′ and 5′-TCCAGGGCCTTCACTTCGGTCT-3′, respectively, resulting in an amplicon of 495 bp. PCR conditions were 95 °C for 1 min, 55 °C for 1.5 min and 72 °C for 1 min performed in a 50 μl final volume. Positive and negative controls were obtained from a patient with acute infectious mononucleosis and from a patient serologically negative for EBV infection, respectively.

4. Results

4.1. Clinical and hematological features

Two patients were young (8 and 18 years) and one was elderly (68 years). All patients were males. All patients had adenopathy, the spleen was enlarged in two cases, the liver in one cases. All cases presented cutaneous infiltration: a 5 cm purpuric subcutaneous nodule on the right upper arm and purpuric infiltrative plaques on the face, neck, chest, back and abdomen in patient 1; purpuric plaques on the face and chest in patient 2; subcutaneous nodules on the right elbow and right wrist in patient 3. Biopsy of cutaneous lesions showed superficial and deep dermal tumor infiltration in two cases (cases 1 and 2) and subcutaneous infiltration in case 3.

Lymph node biopsies were performed in all patients and the histological diagnosis was lymphoblastic lymphomas (WF). Bone marrow biopsies were performed in case 1 and revealed a hypercellular marrow infiltrated with lymphoblastoid cells. Tumor cells were detected in bone marrow (95–100%) and in peripheral blood (46–85%) smears of all cases. Tumors cells were detected in cerebrospinal fluid of case 2. In Leishman-stained smears, tumor cells showed finely distributed nuclear chromatin with nucleoli and a moderate amount of cytoplasm. PAS staining was positive in all cases but the MPO and α-naphthyl-acetate-esterase (ANAE) reactions were negative.

The clinical course was very aggressive in two cases with a survival ranging from 60 to 90 days despite treatment with CHOP (case 1) or CHOP-Bleo and M-BACOD (case 2). On the other hand, patient 3 was treated as acute lymphoblastic leukemia according to the Brazilian protocol for ALL/93 and has been in remission for 7 years.

4.2. Immunophenotype, NK activity, TCR configuration and EB virus genoma

The immunophenotypes determined by flow cytometry were identical concerning $mCD3^-cytCD3^-CD4^{+weak}CD56^+$ $HLA\text{-}DR^+$. In addition, CD2 was expressed in two cases, CD5 in one case and CD7 in all cases (Table 2). The phenotype CD45RA + CD45RO was present in all cases. The expression of CD1, CD8, Tγδ, CD25, CD11b, CD13, CD33, CD14, CD16, CD57, CD122 and TdT was negative. The TCR was in germline configuration in the two cases tested. NK cell activity was demonstrated in one out of two cases tested (case 2) (Table 3). EBV DNA was negative in the two cases tested.

Table 3
Specific cytotoxicity of peripheral blood lymphoid cells against K562 targets

E/F ratio	Case 1	Case 2	Controls ($n = 10$)
160:1	0	34	53.3 ± 9.63[a]
40:1	0	30	29.9 ± 7.07
10:1	0	7	9.2 ± 4.15
2.5:1	0	1.5	2 ± 1.5

E/F: efector/target.
[a] Mean ± S.D.

5. Discussion

We present a series of three cases of $CD3^{-}CD4^{+}CD56^{+}$ blastic NK cell leukemia lymphoma which were diagnosed in the leukemic phase. In contrast with previous reports which described the disease mainly in elderly patients two cases of this study were young (8 and 18 years). In addition, cutaneous disease was present in all cases and the tumor cells were large granular lymphoid cells as observed in the majority of the cases reported in the literature. The study of the immunophenotypic characteristics was facilitated since the three cases presented in leukemic phase. The phenotypes were basically the same $mCD3^{-}cytCD3^{-}CD4^{+weak}CD56^{+}$ $HLA\text{-}DR^{+}$, although the expression of CD2, CD5 and CD7 was not uniform, sometimes changing in different tissues of the same patient. The existence of a normal counterpart of these $CD3^{-}CD4^{+}CD56^{+}$ NK tumor cells has not been definitely established. Recently, Chaperot et al. after the analysis of the malignant cells from seven patients with $CD3^{-}CD4^{+}CD56^{+}$ leukemia suggested that these $CD4^{+}CD56^{+}$ leukemic blast cells are related to plasmacytoid dendritic cells [18]. The three cases of our study also expressed HLA-DR and CD45RA but not CD45RO which are considered to be reminiscent of the phenotype of plasmacytoid dendritic cells. Dunne et al. did not detect any $CD3^{-}CD4^{+}CD56^{+}$ subset in the peripheral blood of 15 normal individuals but demonstrated the presence of 8% of $CD3^{+}CD4^{+}CD56^{+}$ cells [19]. In addition, an infiltration of $CD3^{+}CD4^{+}CD56^{+}$T cytotoxic cells (but not of $CD3^{-}CD4^{+}CD56^{+}$) was found in liver biopsies of patients infected with hepatitis B virus [20], within kidney allografts [21] and in cell lines established from the peripheral blood of multiple sclerosis patients stimulated with myelin basic protein [22]. The negative reactions with ANAE, CD11b and CD14 strongly suggested that the tumor cells were not of the monocytic lineage. The absence of positivity for myeloperoxidase, CD13 and CD33 excluded the diagnosis of myeloid/NK cell precursor acute leukemia [2,23]. NK activity was detected in only one of our cases. To our knowledge cytotoxic activity was not evaluated in the cases previously reported and only the lack of cytotoxic molecules such as TIA-1, granzyme B and perforin was demonstrated in this disease [2,11]. In contrast with aggressive NK cell leukemia [24] and extranodal NK/T cell lymphomas [24], none of our cases was found to be EBV positive despite the use of a sensitive PCR assay. The absence of EBV DNA in tumor cells was also previously described in other $CD3^{-}CD4^{+}CD56^{+}$ blastic leukemia/lymphoma cases [2,4,8,9,11–15].

These three cases have characteristics similar to those previously described as $CD3^{-}CD4^{+}CD56^{+}$ blastic NK leukemia/lymphoma [2,26] or as primary cutaneous $CD4^{+}CD56^{+}$ hematolymphoid neoplasm [11]. These findings strongly support the existence of a distinct clinicopathologic entity within the group of NK malignancies not related to EBV.

For further reading see [25].

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