Flow cytometry characterization of leukemic phase of nasal NK/T-cell lymphoma in tumor biopsies and peripheral blood

We report the findings of the immunophenotypic profile of three cases of nasal T/NK cell lymphoma in leukemic phase. Flow cytometry analysis was carried out using cell suspensions of tumor nasal biopsies and peripheral blood. Tumor samples were composed by a mixture of a predominant subset of medium-size true NK cytCD3ε-, sCD3ε-, CD56+ cells mixed with a minor subset of medium-size T/NK sCD3ε+, CD56+ cells. Both subsets were also detected in peripheral blood. In addition, an infiltration of small-size sCD3ε+, CD56- normal T lymphocytes was also present.

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Marrow and blood involvement is uncommon in nasal CD56+CD3- NK lymphomas and may be seen as a terminal event[1-3]. This rare clinical course is different from aggressive NK-cell leukemia which is more frequent in teenagers and young adults, presents a leukemic blood picture associated with hepatosplenomegaly, anemia, neutropenia, thrombocytopenia and a very aggressive clinical course [3]. The immunophenotipic profile of nasal NK/T cell lymphoma is mainly derived from immunohistochemistry studies in tumor biopsies and is still controversial, due to the infiltration by reactional T-lymphocytes hampering its precise definition[4-8].

In order to clarify the expression of T and NK cell markers we studied by flow cytometry the peripheral blood of three patients (one 33-year old male and a 39 and 73-year old females) with presentation in leukemic phase. In addition, we analyzed cell suspensions prepared from tumor biopsies of two of these cases. All patients complained of nasal obstruction for four to 12 months. Only one patient had lymph nodes enlargement (Table 1). The histological and immunohistochemical features of the nasal masses showed a diffuse lymphomatous infiltration of CD3+, CD56+ cells with an angiocentric and angiodestructive pattern and areas of coagulative necrosis. Granzyme was positive in all cases. Perforin and TIA-1 was tested in two cases and were positive in both (cases 1 and 3). Tumor cells were detected in bone marrow aspirates (representing 10, 7 and 12% of nucleated cells, respectively) and in peripheral blood (28, 50 and 41%) smears and showed a lymphoplasmacytic morphology with a mixture of medium-sized large granular (LG) and agranular cells in two cases and predominantly medium-sized LG in another. Nucleoli were inconpiscous or small. Tumor cells were detected in cerebrospinal fluid of one patient. Hemophagocytosis was observed in bone marrow smears of 2 cases. Epstein-Barr virus encoded latent membrane protein determined by immunohistochemistry in nasal tumor biopsies using the LMP monoclonal antibody was positive in all cases. DNA analysis of the junctional sequences of TCRγ and TCRδ rearrangements were studied by PCR9 and the gene was in germ-line configuration in all cases. The immunophenotipic profile of peripheral blood determined by three color flow cytometry was facilitated since all cases were studied in leukemic phase. In addition, in two cases we also studied nasal cell suspensions prepared by teasing out the tumor mass immediately just after the surgical biopsy. The immunophenotype of cell suspensions obtained from nasal biopsies showed in the lymphoid gate the presence of three different subsets: medium-size cytCD3ε-, CD56+ cells (46% in one case and 5% in other), medium-size sCD3ε+, CD56+ (13% and 3,5%) cells and small-size sCD3ε+, CD56- normal lymphocytes (36% and 78%) (Table 2). Flow cytometry analysis also demonstrated these three different subsets in the PB with the predominance of the small size sCD3ε+, CD56- normal T lymphocytes in all cases. The complete profile of the cytCD3ε-, CD56+ subset in peripheral blood was: cytCD3ε-, sCD3ε-, CD2+, CD5-, CD7+, CD8-, TCR-/_-1-, CD56+, CD16+, CD57+, CD161± (+^{ve} in 2 of 3 cases), CD25- considering as positive the expression in 25% or more cells. γ/δ TCR was tested in two cases and was negative in both. On the other hand, the profile of the sCD3e+CD56+ subset was the same except for sCD3ε+, CD5+, CD8+, TCR-/_-1+. Three anti-human killer inhibitory receptors (KIR) were studied: NKB1, CD158a and CD158b. NKB1 and CD158a were negative in both cytCD3ε-CD56+ and sCD3ε+CD56+ subsets in the peripheral blood of the 3 patients. CD158b expression studied only in two patients (cases 2 and 3) due the lack

Table 1. Clinical and hematological characteristics of patients

									PB and BM infiltration (% of abnormal cells)				
Case	*Age/ sex*	*Symptoms*	*Primary site*	*LN*	*Spleen/ Liver*	*Hb g/dL*	*WBC X10^9/L*	*Platelet X10^9/L*	*PB*	*BM*	*Morphology*	*Treatment*	*Clinical Course*
1	39/F	Nasal obstruction and swelling for 4 months	Mass in the right nasal cavity	C, SM, I	0*	13,6	4,9	302	28	10 HP	Large granular lymphoplasmacytic	BFM-T MIFAP	Dead 3,5 yrs Remission for 3 yrs
2	73/F	Nasal obstruction, fever, night sweats and weight loss for 7 months	Mass in nasopharynx, parapharynx and right maxilar sinus	0	0	12,1	7,3	233	50	7 HP	Large granular and agranular lymphoplasmacytic	Rtx, Cyclophosphamide	Dead, 4 months, no remission
3	33/M	Nasal obstruction for 12 months	Mass in the right nasal cavity	0	0	17,7	8,9	224	41	12	Large granular and agranular lymphoplasmacytic	BFM-T, COAP, Rtx	Dead 4 yrs Remission for 3 yrs

*LN: lymph nodes; Hb: hemoglobin; WBC: white blood cells; PB: peripheral blood; BM: bone marrow; C: cervical; SM: submandibular; I: inguinal; HP: hemophagocytosis; Rtx: radiotherapy. * Spleen enlarged only by ultrasound and CT scanning. BFM-T: vincristine, daunorubicin, prednisone, L-asparaginase, cyclophosphamide, mercaptopurine, teniposide, cytarabine and thioguanine. Intrathecal methotrexate, cytarabine and dexamethasone.MIFAP: mitoxantrone, fludarabine, cytarabine and cisplatin*

Table 2. Immunophenotypic profile, TCR configuration and Epstein-Barr virus detection

Patient		*Phenotypic characteristics*	*TCR*	*Epstein-Barr virus*
1	Peripheral blood nasal	27% CD3-CD56+, 6% CD3+CD56+, 36% CD3+CD56- 46% CD3-CD56+, 13% CD3+CD56+, 36% CD3+CD56-	Germ-line	Positive
2	Peripheral blood	38% CD3-CD56+, 3% CD3+CD56+, 51% CD3+CD56-	Germ-line	Positive
3	Peripheral blood nasal	11% CD3-CD56+, 4% CDCD3+CD56-, 68% CD3+CD56- 5% CD3-CD56+, 3,5% CD3+CD56-, 78% CD3+CD56-	Germ-line	Positive

of available samples. In case 2, it was negative in the peripheral blood and bone marrow in both cytCD3ε-CD56+ and sCD3ε+CD56+ subsets. In case 3, CD158b was positive in both sCD3ε+CD56+ and cytCD3ε-CD56+ subsets in bone marrow cells but it was not studied in the peripheral blood due the lack of available samples. The finding of the CD158a-/CD158b+ phenotype in this case confirmed that NK population was neoplastic. The findings of KIR expression in these three cases are in agreement with previous reports which described the expression of CD158a/CD158b only in some cases of nasal lymphomas[10,11]. The complete subset was not determined in nasal biopsies due the paucity of cells. All patients died of disease at four, 42 and 48 months, respectively, despite treatment with radiotherapy and/or chemotherapy.

Therefore, we took advantage of the presence of tumor cells in the peripheral blood in order to perform a comprehensive identification of the immunophenotype. The present findings clarifies the previous discordant results concerning the CD3 expression in tumor cells of nasal lymphomas and demonstrated that in nasal biopsies the lymphoid cells are composed by a mixture of a medium-size true NK cytCD3ε-, sCD3ε-, CD56+ cells mixed with a subset of medium-size T/NK sCD3ε+, CD56+ cells. In addition, a predominant infiltration of small-size sCD3+ε, CD56- normal T lymphocytes was also present in nasal biopsies. Both the T/NK and the T subsets most likely represent the reactive infiltrating cells since they were in germline configuration. In fact, Tao *et al* [5] combining EBER in situ hybridization with immunohistochemistry for cellular markers in nasal biopsies reported that EBV+ cells (putative tumor cells) were CD3_- whereas the EBV- cells were CD3+ and represented the background reactive cells.

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