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Lymphoid subsets in acute myeloid leukemias: increased number of cells with NK phenotype and normal T-cell distribution

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Summary. Natural killer (NK) and T subsets were analyzed with appropriate dual labeling by flow cytometry in peripheral blood (PB) (66 cases) and bone marrow (BM) (55 cases) from patients with de novo AML in order to determine: (a) their distribution at diagnosis, (b) the correlation between PB and BM in NK subpopulations, (c) their relationship with the clinical and hematological disease characteristics, and (d) the changes occurring upon achieving complete remission (CR). NK cells defined by the expression of CD56 in the absence of CD3 were significantly increased at diagnosis and their levels in PB correlated with those of BM. By contrast, NK subsets defined by CD16 expression (CD16+ CD2+ and CD16+ CD2− NK-cell subsets) as well as T lymphocytes with NK activity (CD56+ CD3+), although increased in PB, displayed normal levels in BM. An additional observation of interest was the expansion of an immature NK population lacking CD16 Ag expression (CD56+ CD16−). AML cases were divided into two groups according to the absolute number of NK cells in PB; patients with the highest levels showed an increased proportion of blast cells in PB ($p=0.01$), monocytic subtypes ($p=0.03$), and expression of CD11b, CD14, and CD4 antigens ($p=0.05$). Infections at diagnosis were not related to the level of NK cells. In 19 patients who achieved complete remission the number of CD56+CD3− cells tended to be reduced to within the normal range. Other T-cell populations, including the CD4 naive and memory cells, were also explored, their distribution being normal in the PB of AML patients. By contrast, the cytotoxic subset CD8+/CD57+ was significantly increased ($p<0.001$). These data point to the existence of marked alterations of NK cells in AML patients, possibly reflecting a host-tumor immunological interaction.

Key words: NK cells – T cells – AML – Acute myeloid leukemia

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Introduction

Natural-killer cells are characterized by their capacity to lyse virus-infected cells and tumor cell targets without major histocompatibility complex (MHC) restriction [14, 28], protecting against both spontaneously arising and experimentally induced neoplasms [27–29]. In spite of the importance of this cell population, it remains to be discovered whether NK cells represent a cohort of cells from different hematopoietic lineages or a homogeneous lineage-restricted cell population [28, 29].

Immunophenotypical studies have demonstrated that cells with NK activity express a heterogeneous array of surface antigens [28, 29]. The most potent NK cells are a non-T-cell population (CD3−, TCR−) that express both the CD16 and CD56 antigens. Together with this population there is a minor subset with a less efficient NK activity corresponding to T (CD3+) lymphocytes, which co-express the CD56 and CD57 Ags but lack CD16 [13]. Lanier et al. [14] have designated these latter cells as non-MHC-restricted cytotoxic T lymphocytes. Several observations indicate that NK cells play a role in the control of leukemia: (a) NK function is altered in leukemic patients [3, 4, 8, 18]; (b) NK activity tends to decrease in individual patients who subsequently relapse [25, 37]; and (c) in animal models, transfer of NK cells may protect against induced leukemia [26]. It is already known that some human malignant cells (non-Hodgkin's lymphoma, Hodgkin's lymphoma, renal cell carcinoma, colon carcinoma, and melanoma) are susceptible to lysis by lymphokine-activated killer (LAK) cells either in vitro [10] and/or in vivo [30, 40] and that, in vivo, these killer cells play a role in the host's defense against ANLL [5, 8, 17, 20, 34]. However, it seems that blast cells from acute myeloid leukemia are usually resistant to NK-mediated killing [1, 36], although the stimulation of effector cells with interleukin-2 may significantly increase the percentage of specific ^{51}Cr release in cytotoxicity assays [16, 24].

In the present study we have analyzed several lymphocyte subsets defined by NK-associated antigens as well as T-cell subsets in AML patients in order to determine: (a) their distribution at diagnosis, (b) the correlation between peripheral blood (PB) and bone marrow (BM) NK-cell subpopulations, (c) their relationship with clinical and hematological disease characteristics, and (d) their changes when complete remission (CR) is achieved.

Materials and methods

Patients and controls

T-lymphoid and NK-associated antigens were explored in peripheral blood (PB) (66 cases) and bone marrow (BM) (55 cases) samples from patients with de novo acute myeloid leukemia (AML) diagnosed according to the FAB criteria. In each patient the most relevant clinical and hematological diseases characteristics documented at diagnosis were evaluated for correlation with lymphoid subsets. Thirty sex- and age-matched healthy volunteers were used as controls for peripheral blood (PB) studies, and ten volunteers undergoing orthopedic surgery were used for bone marrow (BM) studies. In these cases BM aspiration was performed immediately after the induction of anesthesia. Informed consent was obtained from both the AML patients and the volunteers.

Immunophenotypical studies

T-lymphoid and NK-associated antigens were explored by direct immunofluorescence with flow cytometry, as previously described [12, 32]. The panel of monoclonal antibodies (MoAb) used in the PB samples included: CD3 (Leu 4), CD2 (Leu 5b), CD4 (Leu 3), CD8 (Leu 2), CD56 (Leu 19), CD16 (Leu 11c), CD29 (4B4), CD45RA (Leu 18), and CD57 (Leu 7). Simultaneous stainings were systematically used to analyze the following antigenic co-expressions: CD3/CD56, CD2/CD16, CD4/CD29, CD4/CD45RA, and CD8/CD57. For BM studies, the following combinations of MoAb were employed: CD3/CD56 and CD2/CD16. Irrelevant isotype-matched MoAb were employed as negative controls [33].

AML cases in which the blast cells had a light scatter distribution pattern [forward scatter (FSC)/side scatter (SSC)] that did not permit a clear distinction from lymphoid cells and also expressed the CD56 and/or CD16 antigens were not included in the study.

Owing to the possible contamination of the "lymphoid" gate by erythroblasts and blast cells, the proportion of each lymphoid subset ("x") was corrected according to the total number of T lymphocytes (CD3+/CD56− and CD3+/CD56+) and NK cells (CD3−/CD56+) present in the cell gate analyzed, using the following equation:

$$\%\ \text{"x"} = \frac{x}{(CD3+/CD56-)+(CD3+/CD56+)+(CD3-/CD56+)}$$

The absolute numbers of the different subpopulations analyzed ("x") were calculated from the total number of PB leukocytes, the proportion of FSC/SSC lymphocyte region gate, and the percentage of positive cells within the gate:

Abs. count "x" = Total leukocytes x % gate x % positive cells

Statistical methods

To estimate the significance of the differences between means the Mann-Whitney U test (BMDP 3S program) was used. The T- and NK-cell populations were correlated with presenting clinical features by means of the chi-square test (BMDP 4F program). Survival curves were plotted according to the method of Kaplan and Meier and compared statistically using the Mantel-Cox and Breslow tests (BMDP 1L program) [7].

Results

The expression of NK-associated antigens (CD56 and CD16) was significantly increased ($p<0.001$) in the PB of AML patients, both in relative and in absolute numbers. Moreover, the augmented expression of CD56 was due to a uniform expansion of the two major CD56+ subsets: the typical CD56+ CD3− NK cells and the CD3+ CD56+ T lymphocytes. Within the CD16+ cells there was also a parallel expansion of both the CD16+CD2− and the CD16+CD2+ NK cell subsets (Table 1).

A significant increase in the percentage of CD56+ cells ($p=0.01$) was also observed in the BM of AML patients, although in this case it was due only to the CD56+ CD3− subset, while the level of CD56+CD3+ cells was normal. By contrast, the distribution of CD16 cells did not significantly differ from that of normal BM samples (Table 2). Upon correlating the relative number of NK cells in BM and PB in the 35 cases in which they were analyzed simultaneously, a significant correlation was found for the CD56+CD3− cells (Fig. 1).

Regarding PB T-cell subsets, the number of CD4+ and CD8+ lymphocytes, both in percentage and absolute numbers, were within the normal range; nevertheless, the slight increase of CD8+ cells with a decrease in CD4 cells led to an imbalanced CD4/CD8 ratio ($p=0.02$). The distribution of the two major CD4 sub-

Table 1. Expression of NK-associated Ag in PB

	AML patients $n=66$	Healthy controls $n=30$	p value
CD56+ CD3−	23 ± 17 (1000 ± 2400)	8 ± 4) (100 ± 70)	0.001
CD56+ CD3+	16 ± 18 (500 ± 1200)	4 ± 1 (50 ± 30)	0.001
Total CD56+	38 ± 23 (1570 ± 3400)	11 ± 5 (170 ± 90)	0.001
CD16+ CD2+	12 ± 12 (400 ± 600)	4 ± 4 (60 ± 40)	0.001
CD16+ CD2−	9 ± 12 (500 ± 1500)	2 ± 2 (20 ± 20)	0.001
Total CD16+	22 ± 18 (900 ± 1800)	6 ± 6 (90 ± 60)	0.001

Results expressed as mean ± SD of relative (top lines) and absolute numbers (in brackets)
Absolute numbers expressed as cells × 10^6/l

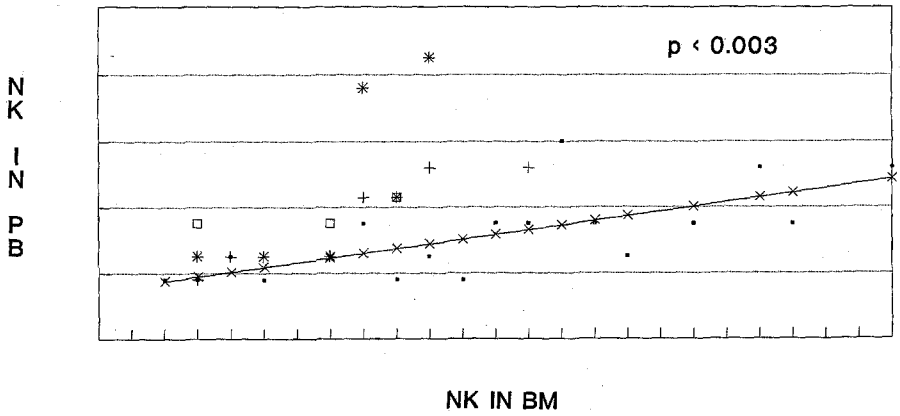

Fig. 1. Correlation between peripheral blood (*PB*) and bone marrow (*BM*) content of natural killer (*NK*) cells (CD56+ CD3−)

Table 2. Expression of NK-associated Ag in BM

	AML patients $n=55$	Healthy controls $n=10$	p value
CD56+ CD3−	22 ± 16	7 ± 5	0.01
CD56+ CD3+	12 ± 11	9 ± 5	NS
Total CD56+	34 ± 19	16 ± 9	0.01
CD16+ CD2+	9 ± 7	9 ± 3	NS
CD16+ CD2−	9 ± 11	5 ± 3	NS
Total CD16+	19 ± 14	14 ± 7	NS

Results expressed as mean ± SD of relative numbers

Table 3. T-cell subsets in PB

	AML patients $n=66$	Healthy controls $n=30$	p value
Total CD4+	49 ± 18 (2000 ± 6900)	56 ± 13 (800 ± 300)	NS
Naive CD4+ CD45RA+	20 ± 13 (1200 ± 5600)	22 ± 12 (300 ± 100)	NS
Memory CD4+ CD29+	28 ± 10 (1300 ± 4700)	31 ± 7 (400 ± 100)	NS
Total CD8+	46 ± 32 (1900 ± 4900)	36 ± 9 (500 ± 200)	NS
Cytotoxic CD8+ CD57+	12 ± 12 (800 ± 2400)	4 ± 4 (50 ± 30)	0.001
CD4/CD8	1.4 ± 0.9	1.7 ± 0.8	0.02

Results expressed as mean ± SD of relative (top lines) and absolute numbers (in brackets)
Absolute numbers expressed as cells × 10^6/l

sets – memory (CD4+/CD29+) and naive (CD4+/CD45RA+) – was also normal, while within the CD8+ cells CD8+CD57+ supressor-cytotoxic lymphocytes were significantly increased ($p=0.001$) both in relative and absolute numbers (Table 3). This latter increase was due mainly to the $CD8^{bright}$ cells, which correspond to T lymphocytes.

The AML patients were divided into two groups according to the absolute number of NK cells in PB (CD56+ cells higher or lower than 240×10^6/l). Patients displaying higher levels of NK cells showed an increased proportion of blast cells in PB ($p=0.04$), monocytic subtypes – assessed both by morphological and immunological criteria – ($p=0.003$), together with a greater expression of CD11b ($p=0.003$), CD14 ($p=0.02$), and CD4 ($p=0.10$) antigens. Although the differences were not statistically significant in this group of patients, overall survival was also higher ($p=0.07$) (Table 4). In 19 patients, study of the NK populations in BM was repeated once the patients had achieved CR, with the finding of a decrease in the numbers of NK CD56+ CD3− cells to levels similar to those of the controls. The remaining NK-cell subsets analyzed, which, as described above, were within the normal range at diagnosis, remained unchanged when CR was achieved (Fig. 2).

Table 4. Relationship between the number of NK cells and the clinical and hematological characteristics

	Numbers of CD56+CD3− cells		
	$<240\times10^6$/l $n=32$ (%)	$\geq240\times10^6$/l $n=32$ (%)	
% Blast cells in PB[a]	47 ± 30	62 ± 27	0.04
Monocytic phenotype	3/32 (9)	13/32 /40)	0.003
CD11b	13/32 (40)	25/32 (77)	0.003
CD14	4/31 (13)	12/31 (39)	0.02
CD4	15/31 (48)	22/32 (69)	0.10
% CR	(58)	(66)	NS
Survival (months)	5	10	0.07

[a] Results expressed as mean ± SD

Discussion

Analysis of lymphoid subsets has proved to be of relevance in a wide range of clinical disorders, contributing both to a better understanding of their immunobiology [11, 12, 19, 31] and to better management of the course of these diseases [11, 31]; HIV infection is the most representative example of this.

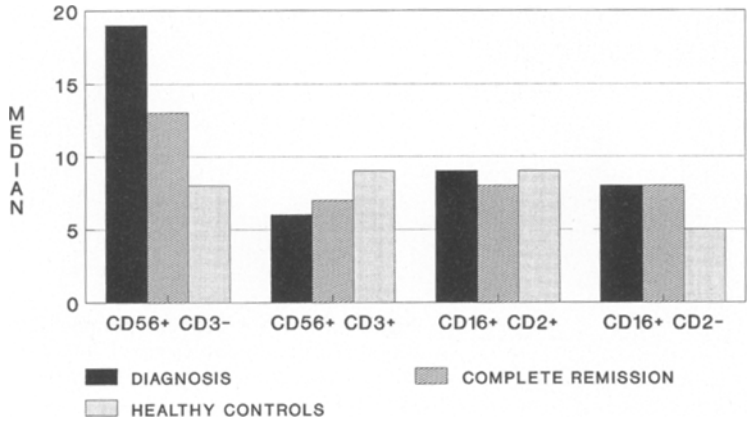

Fig. 2. Natural killer (*NK*) cells in bone marrow (*BM*): diagnosis vs complete remission

Few studies have addressed T-cell subsets in AML patients at diagnosis [21]. At present, most of the available information refers to the immune reconstitution following bone marrow transplantation. The present study shows that the two major T-cell subsets (CD4, CD8), as well as the CD4 subpopulations (naive and memory), lie within the normal range, both in relative and absolute numbers, in AML patients at diagnosis. By contrast, there is a significant increase in CD8+/CD57+ supressor/cytotoxic lymphocyte subsets, probably reflecting host-tumor immunological interactions.

The availability of appropriate MoAb that recognize Ags associated with cells with NK activity allows the definition of a cell type rather than a cellular function. At present very few studies have focused on the number of NK cells in AML. Moreover, most of these studies, performed after IL-2 stimulation, have yielded quite variable results, ranging from decreased [35] to normal [2] or increased [16] levels of NK cells. In the present study, using double staining to assess different NK-cell subsets, we have found that NK cells defined by the expression of CD56 in the absence of CD3 were significantly increased in AML patients at diagnosis, and that their levels in PB correlated with those in BM. By contrast, NK subsets defined by CD16 expression (CD16+ CD2+ and CD16+ CD2− NK-cell subsets) as well as T lymphocytes with NK activity (CD56+ CD3+), although increased in PB, displayed normal levels in BM. Moreover, sequential studies performed in our series show that the number of CD56+CD3− cells tended to be within the normal range upon achievement of CR.

An additional observation of interest is that the number of NK cells (CD3−) expressing the CD56 Ag was always higher than that of lymphocytes defined by CD16 positivity. These results indicate that in AML patients an expansion occurs in an NK population lacking CD16 Ag expression (CD56+ CD16−); these cells have been considered to be the most immature NK subset [22]. In addition, the increase in CD16+ NK-cells in PB without a similar expansion in BM suggests that probably not all NK-cell subsets display the same tissue-homing pattern. The present study also points to certain correlations between the number of NK cells and several hematological and immunophenotypical disease characteristics.

It has been suggested that some cell populations may play an important role in protection against proliferation of neoplastic cells [28, 29]. The increased levels of NK cells detected in the present study would support this hypothesis. The paradox between the high number of NK cells found by us and the decreased NK activity previously reported in AML patients suggests the existence of a defective cell function. This alteration can be attributed, at least in part, to a functional impairment of NK cells to produce natural-killer cytotoxic factor (NKCF) [41], which has been identified as IL-12 [23], and to a possible immunosuppressive effect of a serum factor elaborated by leukemic myeloblasts [15]. In addition, although these patients might have a normal NK function, it may not be efficient, owing to a reduced susceptibility of the blast cells to lysis by autologous NK or LAK effectors [1, 2, 38]. Recently, it has been shown that CD8+ CD57+ lymphocytes may release a factor that suppresses killer activity [6]. Since we have detected a significant increase in this cell subset in AML patients, it is possible that these lymphocytes may be at least partially responsible for the defective NK function of AML patients. However, an alternative possibility that may help to explain this paradox is that the discrepancy between NK-cell numbers and NK activity in AML patients is not a real one, but rather related to technical problems. Thus, reduced blood cell NK activity could be due largely to a "dilution" effect of NK cells by leukemic cells in the mononuclear fraction, leading to a low effector/target cell ratio [28]. The reported normalization of NK and LAK activity upon achievement of CR [9, 39] would support, at least partially, these hypothesis.

These data point to the existence of marked alterations of NK cells in AML patients, possibly reflecting a host-tumor immunological interaction.

References

1. Adler A, Albo V, Blatt J, Whiteside TL, Herberman RB (1989) Interleukin-2 induction of lymphokine-activated killer (LAK) activity in the peripheral blood and bone marrow of acute leukemia patients. II. Feasibility of LAK generation in children with active disease and in remission. Blood 74:1690–1697
2. Adler A, Chervenick PA, Whiteside TL, Lotzová E, Herberman RB (1988) Interleukin-2 induction of lymphokine-activated killer (LAK) activity in the peripheral blood and bone marrow of acute leukemia patients. I. Feasibility of LAK generation in adult patients with active disease and in remission. Blood 71:709–716
3. Adler A, Chervenick P, Whiteside T, Lotzová E, Herberman RB (1987) IL-2 mediated in vitro stimulation of killer cells derived from peripheral blood (PB)/bone marrow (BM) of acute leukemia patients. Fed Proc 46:1509 (abstr 6957)
4. Afify ZA, Abdel-Mageed A, Findley HW, Ragab AH (1990) Cytotoxicity of purified Leu 19+ cells from the peripheral blood of children with acute lymphocytic leukemia. Cancer 66:469–473
5. Archimbaud E, Bailly M, Doré JF (1991) Inducibility of lymphokine-activated killer (LAK) cells in patients with acute myelogenous leukaemia in complete remission and its clinical relevance. Br J Haematol 77:328–334
6. Autran B, Leblond V, Sadat-Sowti B, Lefranc E, Got P, Sutton L, Binet JL, Debre P (1991) A soluble factor release by CD8+CD57+ lymphocytes from bone marrow transplanted patients inhibits cell-mediated cytolysis. Blood 77:2237–2241
7. Dixon WJ (1983) (Biomedical-Data Package): Statistical software. University of California, Berkeley
8. Foa R, Fierro MT, Cesano A, Guarini A, Bonferroni M, Raspadori D, Miniero R, Lauria F, Gavosto F (1991) Defective lymphokine-activated cell generation and activity in acute leukemia patients with active disease. Blood 78:1041–1046
9. Foa R, Guarini A, Gillio-Tos A, Cardona S, Fierro MT, Meloni G, Tosti S, Mandelli F, Gavosto F (1991) Peripheral blood and bone marrow immunophenotypic and functional modifications induced in acute leukemia patients treated with interleukin-2: evidence of in vitro lymphokine-activated killer cell generation. Cancer Res 51:964–968
10. Gambacorti-Passerini C, Rivoltini L, Supino R, Mariani M, Parmiani G (1988) Differential lysis of melanoma clones by autologous recombinant interleukin-2 activated lymphocytes. Relationship with spontaneous resistance to doxorubicin (Dx). Int J Cancer 42:544–548
11. Giorgi JV, Kesson AM, Chou CC (1992) Immunodeficiency and infectious diseases. In: Rose NR, de Macario EC, Fahey JL, Freidman H, Penn GM (eds) Manual of clinical and laboratory immunology. Section D: Immune cell phenotyping by flow cytometry. Amer Soc Microbiol, Washington DC, pp 174–181
12. González M, San Miguel JF, Gascón A, Moro MJ, Hernández JM, Ortega F, Jiménez R, Guerras L, Romero M, Casanova F, Sanz MA, Portero JA, Orfao A (1992) Increased expression of natural killer-associated and activation antigens in multiple myeloma. Am J Hematol 39:84–89
13. Lanier LL, Phillips JH (1987) Evidence for three types of human cytotoxic lymphocyte. Immunol Today 7:132–135
14. Lanier LL, Phillips JH, Hackett J, Tutt M, Kuman V (1986) Natural killer cells: definition of a cell type rather than a function. J Immunol 137:2735–2739
15. Lim SH, Worman CP, Jewell A, Goldstone AH (1991) Production of tumor-derived suppressor factor in patients with acute myeloid leukemia. Leuk Res 15:263–268
16. Lotzová E, Savary CA, Schachnr JR, Huh JO, McCredie K (1991) Generation of cytotoxic NK cells in peripheral blood and bone marrow of patients with acute myelogenous leukemia after continuous infusion with recombinant interleukin-2. Am J Hematol 37:88–99
17. Lotzová E, Savary CA, Totpal K, Schachner J, Lichtiger B, McCredie KB, Freireich EJ (1991) Highly oncolytic adherent lymphocytes: therapeutic relevance for leukemia. Leuk Res 15:245–254
18. Lotzová E, McCredie KB, Muesse L, Dicke KA, Freireich EJ (1989) Natural killer cells in man: their possible involvement in leukemia and bone marrow transplantation. In: Baum SJ, Ledney (eds) Experimental hematology today. Springer, Berlin Heidelberg New York, p 207
19. Lum LG, Ueda M (1992) Immunodeficiency and the role of suppressor cells after the bone marrow transplantation. Clin Immunol Immunopathol 63:103–109
20. Maraninchi D, Blaise D, Viens P, Brandely M, Olive D, López M, Sainty D, Marit G, Stoppa AM, Reiffers J et al. (1991) High-dose recombinant interleukin-2 and acute myeloid leukemias in relapse. Blood 78:2182–2187
21. Milosevic D, Marinkovic M, Suvajdzic N, Spuzic I (1991) Determination of T lymphocyte subpopulations in malignant lymphoproliferative diseases. Glas Srp Akad Nauka [Med] 41:105–111
22. Nagler A, Lainer LL, Cwirla S, Phillips JH (1989) Comparative studies of human FcR III-positive and -negative natural killer cells. J Immunol 143:3183–3191
23. Naume B, Gately M, Espevik T (1992) A comparative study of IL-12 (cytotoxic lymphocyte maturation factor), IL-2 and IL-7-induced effects on immunomagnetically purified CD56+ NK cells. J Immunol 148:2429–2436
24. Palucka AK, Porwit A, Reizenstein P (1991) Resistance of leukemic blasts to lymphokine-activated killer (LAK)-mediated cytotoxicity is not related to their adhesion properties. Eur J Haematol 47:123–127
25. Pizzolo G, Trentin L, Vinante F, Agostini C, Zambello R, Masciarelli M, Feruglio C, Dazzi F, Todeschini G, Chilosi M, et al. (1988) Natural killer cell function and lymphoid subpopulations in acute non-lymphoblastic leukemia in complete remission. Br J Cancer 58:368–372
26. Pollack SB (1983) In vivo functions of natural killer cells. Surv Synth Pathol Res 2:93–98
27. Rees RC (1990) MHC restricted and non-restricted killer lymphocytes. Blood Rev 4:204–210
28. Richards SJ, Scott CS (1992) Human NK cells in health and disease: clinical, functional, phenotypic and DNA genotypic characteristics. Leukemia Lymphoma 7:377–399
29. Robertson MJ, Ritz J (1990) Biology and clinical relevance of human natural killer cells. Blood 76:2421–2438
30. Rosenberg SA, Lotze MT, Mull LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson GN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 316:889–897
31. San Miguel JF, Caballero MD, González M (1985) T-cell subpopulations in patients with monoclonal gammopathies: essential monoclonal gammopathy, multiple myeloma and Waldeström's macroglobulinemia. Am J Haematol 20:267–273
32. San Miguel JF, Hernández JM, González-Sarmiento R, González M, Sánchez I, Orfao A, Cañizo MC, López-Borrasca A (1991) Acute leukemia after primary myelodisplastic syndrome: immunophenotyping, genotypic and clinical characteristics. Blood 78:768–774
33. San Miguel JF, Ojeda E, González M, Orfao A, Cañizo MC, Sánchez I, López-Borrassca A (1989) Prognostic value of immunological markers in acute myeloblastic leukemia. Leukemia 3:108–111
34. Savary CA, Lotzová E (1989) Natural killer cell-mediated inhibition of growth myeloid and lymphoid clonogenic leukemias. Exp Hematol 17:183–187
35. Sorskaar D, Forre O, Tjonneland S, Lie SO (1988) Altered

natural killer cell activity in childhood acute non-lymphoid leukaemia. Augmented natural killer activity and cells expressing the Leu 7 and Leu 11b (CD16) markers in the bone marrow of children in remission. Scand J Immunol 28:11–17

36. Tratkiewicz JA, Szer J, Boyd RL (1988) Are lymphokine-activated killer cells a possible form of leukemia immunotherapy? Adv Exp Med Biol 237:447–450
37. Tratkiewicz JA, Szer J (1990) Loss of natural killer activity as an indicator of relapse in acute leukemia. Clin Exp Immunol 80:241–246
38. Tratkiewicz JA, Szer J, Boyd RL (1990) Lymphokine activated killer cytotoxicity against leukaemic blast cells. Clin Exp Immunol 80:94–99
39. Trentin L, Pizzolo G, Ferugio C, Zambello R, Masciarelli M, Bulian P, Agostini C, Vinante F, Zanotti R, Semenzato G (1989) Functional analysis of cytotoxic cells in patients with acute nonlymphoblastic leukemia in complete remission. Cancer 64:667–672
40. Weber JS, Yang JC, Topalian SL, Schwartzentruber DJ, White DE, Rosenberg SA (1992) The use of interleukin-2 and lymphokine-activated killer cells for the treatment of patients with non-Hodgkin's lymphoma. J Clin Oncol 10:33–40
41. Yamada S, Komiyama A (1991) Decrease in number of CD16 (Leu 11)+ CD45RA(2h4)+ cells and defective production of natural killer cytotoxic factor in childhood acute lymphoblastic leukemia. Leuk Res 15:785–790