Proliferation of myeloid lineage cells and apoptosis of lymphoblastic leukemic cells induced by short-course high-dose methylprednisolone in patients with acute lymphoblastic leukemia

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SUMMARY: Yıldıran A, Erduran E, Tekelioğlu Y, Dilber E, Gedik Y. Proliferation of myeloid lineage cells and apoptosis of lymphoblastic leukemic cells induced by short-course high-dose methylprednisolone in patients with acute lymphoblastic leukemia. Turk J Pediatr 2002; 44: 116-121.

In this paper, we investigated the effects of short-course high-dose methylprednisolone (HDMP) treatment on the proliferation of myeloid lineage cells and on apoptosis of blast cells in eight children with acute lymphoblastic leukemia (ALL). The patients were given the HDMP treatment (30 mg/kg/d, perorally) before 9:00 a.m. for seven days. Bone marrow (BM) aspiration was done at days 0 and 3 of the HDMP treatment in all patients and at the 7th day of the HDMP treatment in six patients. Bone marrow blast cells had gradually decreased after the HDMP treatment by the 7th day. There were statistically significant differences between the mean percentages of BM blast cells at days 0 and 3, days 0 and 7, and at days 3 and 7 ($p<0.05$). The mean percentages of blast cell apoptosis at the 3rd day was significantly higher than at days 0 and 7, and apoptosis at day 0 was significantly lower than at the 7th day ($p<0.05$). The mean percentages of BM myeloid lineage cells at the 7th day was significantly higher than at days 0 and 3 ($p<0.05$), and the mean percentage at day 0 was significantly lower than at the 3rd day ($p<0.05$).

These findings indicate that short-course HDMP treatment causes apoptosis on lymphoblasts and increases the proliferation of myeloid lineage cells in children with ALL.

Key words: acute lymphoblastic leukemia, short-course high-dose methylprednisolone, proliferation of myeloid lineage cells, apoptosis of lymphoblastic leukemic cells.

High-dose methylprednisolone (HDMP) is used in the treatment of acute lymphoblastic leukemia (ALL) during remission induction chemotherapy[1-3]. HDMP during remission induction chemotherapy improves long-term event-free survival (EFS), especially for high-risk patients with ALL[1]. HDMP also causes acceleration of leukocyte recovery in children with ALL[4]. It was suggested that this effect of HDMP could be due to the increase of the serum granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) levels[5]. On the other hand, HDMP causes apoptosis and differentiation of leukemic cells in patients with acute myeloblastic leukemia (AML)[6-10]. In this study, proliferation of myeloid lineage cells and apoptosis of blast cells induced by short-course HDMP was evaluated in patients with ALL.

Material and Methods

Eight patients with ALL were enrolled in the study with consent of their parents.

All patients received HDMP in a dose of 30 mg/kg/d perorally before 9:00 a.m. for seven days. Bone marrow (BM) aspiration was done at days 0 and 3 of the HDMP treatment in all patients and at the 7th day of the HDMP treatment in six patients for determination of the myeloid lineage cell proliferation and apoptosis of the blast cells. Flow cytometric analyses of BM aspiration materials were done at diagnosis, and CD_2, CD_3, CD_7, CD_{10} (CALLA), CD_{13}, CD_{14}, CD_{19}, CD_{20}, CD_{22}, CD_{33},

CD_{45}, HLA DR, and MPO monoclonal antibodies were used for the phenotyping of blast cells and of the myeloid population.

Bone marrow smears were stained with Wright's dye and 200 cells of bone marrow were counted under the light microscope for each patient. Differential counts of bone marrow cells were calculated as percentages. Apoptosis of the blast cells was determined by flow cytometric analysis. Two parents did not permit BM aspiration at the 7th day. Patients were treated with only HDMP 30/mg/kg/d for seven days, after which HDMP was given 20 mg/kg/d for seven days, and thereafter at 20 mg/kg/alternate day for 14 days in addition to vincristin (0.05 mg/kg in a week for 4 weeks), L-asparaginase (200 u/kg 9 times in 4 weeks), daunorubicin (1 mg/kg 3 times in 4 weeks), and cytosine arabinoside (10 mg/kg 3 times in 4 weeks) as remission induction treatment.

No side effects occurred from the administration of HDMP except for cushingoid appearance and weight gain in all patients.

Separation of Blast Cells and DNA Analysis

Obtained BM aspiration samples at days 0, 3 and 7 of the HDMP treatment were drawn into the tubes with EDTA. Lymphoblasts were obtained by lymphocyte separation medium (Gibco BRL 13010-012). 100 µl aliquots of lymphoblasts were put into the tubes and fixed in DNA prep (Coulter Epics Leukocytes Preparation Workstation) using DNA prepstain (Coulter PN 6604451) and DNA prep LPR (Coulter PN 6604454) kits. All tubes were kept at room temperature in a dark place for 20 minutes. Then, the tubes were treated with Coulter Epics Elite ESP Flow-cytometry. DNA analyses were done using Multicycle AV software (Advanced Version Cell Cycle Analysis, Phoenix Flow Systems, San Diego). Data on cell cycle (G_0/G_1, G_2/M and S phases) cytometry histograms of blast cells displayed a peak less than 2n DNA (Sub G_1), which generally accepted as an indication of cells undergoing apoptosis[11,12].

The proliferation of myeloid lineage cells was determined on gating of forward scatter and PMT_1 parameters in the same software. Gating on flow cytometry was performed according to total mononuclear cells indicating CD_{45} positivity.

Statistical Analysis

The percentages of blast cells of BM and apoptosis of blast cells were calculated as arithmetic mean±standard deviation (mean±SD).

The percentages of blast cells and myeloid lineage cells, and blast cell apoptosis were reciprocally compared for days 0, 3 and 7 of the HDMP treatment using Wilcoxon Rank Sum test.

Results

Bone marrow aspiration smears of the patients exhibited L_1 type morphology in four patients, L_2 morphology in two patients and L_3 type morphology in two patients according to French-American-British (FAB) system. According to ALL prognostic criteria, two patients had high-risk criteria and six patients had low-risk criteria[13]. Bone marrow karyotype analyses could not be performed because of technical limitations.

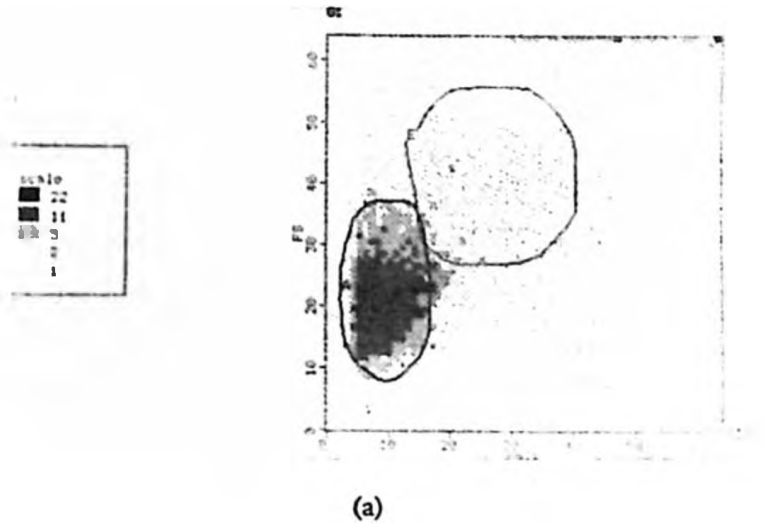
The characteristics of the patients are shown in Table I.

Apoptosis of blast cells was determined by flow cytometric analysis and according to the morphologic appearances of blast cells on light microscope. In addition to their morphologic changes, flow cytometric analysis of BM obtained at days 0, 3 and 7 after HDMP treatment disclosed the new cell population (myeloid lineage cells) (Fig. 1 a-c).

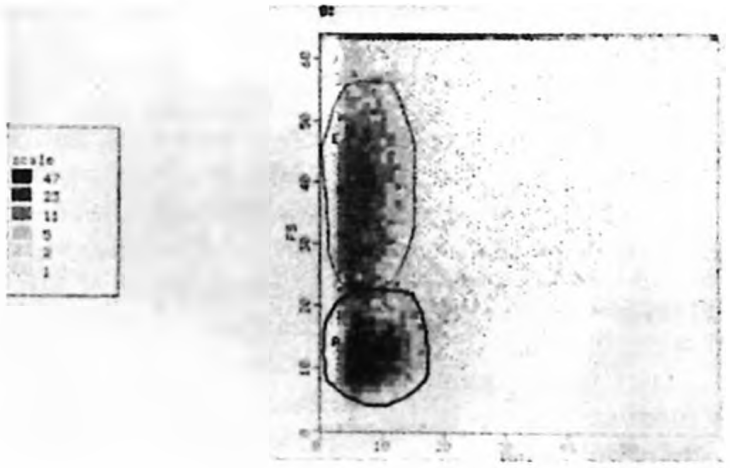
Table I. Patients' characteristics and diagnosis

Patients	Sex	Age	FAB	Leukocytes (/µl)	Diagnosis	Risk group
1	M	5	L3	12,600	B Cell ALL	Low
2	M	7	L1	11,00	B Cell ALL	Low
3	M	7	L1	9,200	Pre-B Cell ALL	Low
4	M	16	L2	38,500	Mixed ALL	High
5	M	7	L1	14,200	Mixed ALL	Low
6	F	8	L3	72,800	B Cell ALL	High
7	M	5	L2	13,700	B Cell ALL	Low
8	M	6	L1	18,900	Pre-B Cell ALL	Low

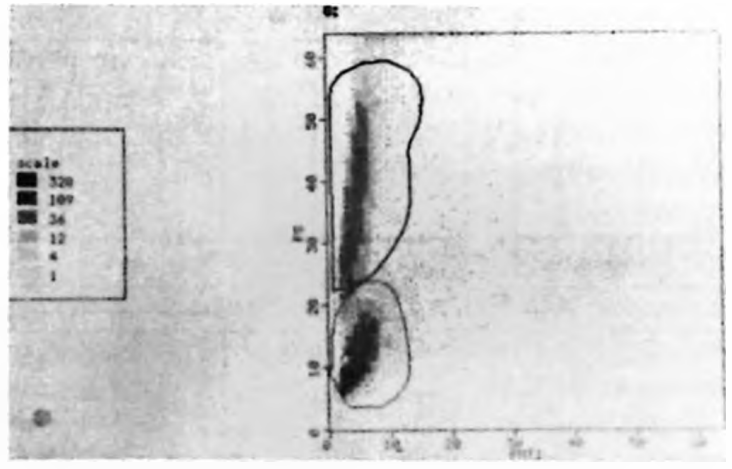
FAB: French-American-British classification. ALL: acute lymphoblastic leukemia.



(a)



(b)



(c)

Fig. 1. A flow cytometric profile of bone marrow of Case 3 on days, a) 0, b) 3 and c) 7 after HDMP treatment. Note the appearance of new cell population (myeloid lineage cells).

Flow cytometry histograms of blast cells displayed a peak less than 2n DNA (Sub G_1), which is indicative of cells undergoing apoptosis, at days 0, 3 and 7 of the HDMP treatment (Fig. 2 a-c).

The percentages of BM blasts, myeloid lineage cells, and apoptosis of blast cells according to flow cytometry histogram of the patients at days 0, 3 and 7 of the HDMP treatment are shown

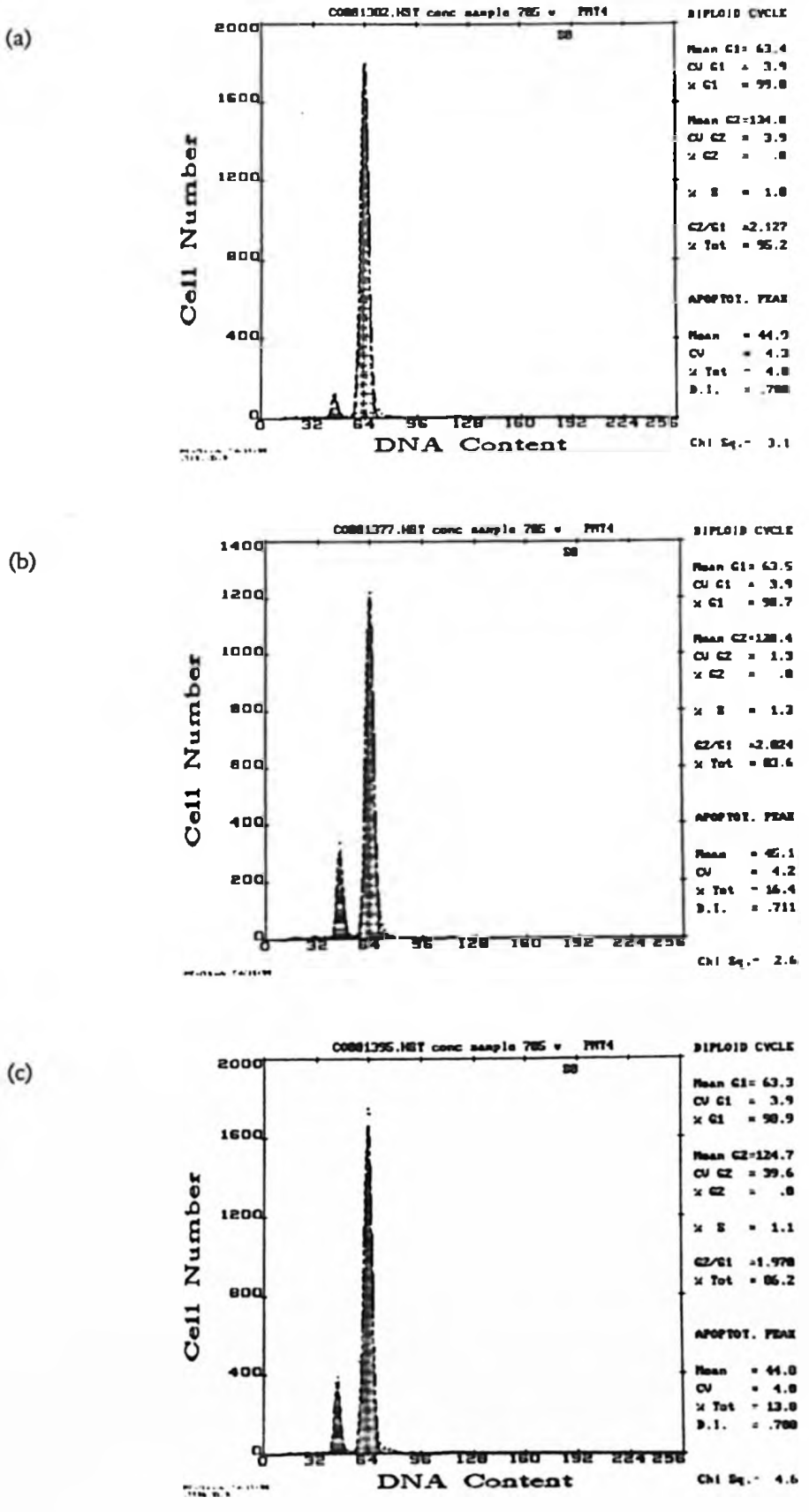


Fig. 2. The flow cytometry histogram of lymphoblasts depicts a peak less than 2n DNA (Sub G_1) on days a) 0, b) 3 and c) 7.

in Table II. BM blast cell percentages decreased and myeloid lineage cells increased by the 7th day, gradually. The percentages of blast apoptosis peaked at day 3.

Table II. Percentages of bone marrow blasts, myeloid lineage cells, and apoptosis of lymphoblasts according to flow cytometry histogram of patients on days 0, 3 and 7 after HDMP treatment

	Day 0			Day 3			Day 7		
Patients	BMB	Apo	Mye	BMB	Apo	Mye	BMB	Apo	Mye
1	95	3.3	1	84	26.3	5	72	17.8	12
2	82	1.6	5	80	15.5	8	66	5.6	11
3	67	2	5	48	7.9	10	26	4.5	32
4	97	4.5	3	85	24	5	18	9.1	5
5	95	1.3	2	83	10.2	4	69	8.7	13
6	93	4.8	3	83	16.4	8	65	13.8	10
7	95	1.7	2	19	3.5	10	ND	ND	ND
8	98	1.6	1	84	17	7	ND	ND	ND
Mean±sd	90.2±10.6[a]	2.6±1.4[b]	2.7±1.5[c]	70.7±24.3[d]	15.1±7.8[e]	7.1±2.2[f]	52.6±24.0[g]	9.9±5.1[h]	13.8±9.3[i]

BMB: bone marrow blasts (%).
Apo : apoptosis (%).
Mye : myeloid cells (%).
ND : not determined.
a-d, a-g, d-g, b-e, b-h, e-h, c-f, f-i, c-i $p<0.05$.

There were statistically significant differences between the mean percentages of BM blast cells at days 0 and 3, days 0 and 7, and days 3 and 7 ($p<0.05$). The mean percentage of blast cell apoptosis at the 3rd day was significantly higher than at days 0 and 7 ($p<0.05$).

There was significant difference between the mean percentages of blast cell apoptosis on days 0 and 7 ($p<0.05$). The mean percentage of bone marrow myeloid lineage cells at the 7th day was significantly higher than at days 0 and 3 ($p<0.05$). There was significant difference between the mean percentages of BM myeloid lineage cells at days 0 and 3 ($p<0.05$). All patients went into remission after remission induction treatment for four weeks.

Discussion

Two different cell deaths are described in vertebrates. Necrosis develops as the result of ischemia and physical and chemical traumas to the cells. In necrosis, all these events are accompanied by early membrane damage and cell disintegration. Apoptosis develops due to physiologic and immunologic factors. The concept of apoptosis as distinguished from necrosis was proposed about 27 years ago by Kerr et al.[14]. When undergoing apoptosis, cells decrease in size and their nuclei condense. Later in the course of apoptosis, the nuclei and the cells become fragmented, and cellular remnants are phagocytosed by macrophages. Fragmentation is double-stranded cleavage of DNA at internucleosomal sites[15,16]. This is determined by showing ladder-formation on gel electrophoresis of DNA extracted from cells.

Cohen et al.[17] suggested that ladder formation does not always indicate apoptosis. A peak less than 2n (Sub G_1) on flow cytometry is accepted as a typical pattern for cells undergoing apoptosis[11,12,18,19].

Fluorescence in situ TUNEL assay, trypan blue assay and flow cytometry detected DNA strand breaks occurring in apoptosis; flow cytometric detection of apoptosis was more sensitive than TUNEL assay[12]. We selected flow cytometry to look for the apoptotic effect of HDMP on lymphoblasts for this reason, and because ladder-formation on gel electrophoresis of DNA extracted from cells is not considered a diagnosis for apoptosis[17].

In this study, it was found that HDMP induced apoptosis of lymphoblastic leukemic cells and caused proliferation of myeloid lineage cells in children with ALL. It was suggested that HDMP caused differentiation and apoptosis of myeloid leukemic cells in children with AML[6-10]. It was found that the apoptotic effect of HDMP on lymphoblasts peaked at the 3rd day and decreased at the 7th day after beginning HDMP treatment ($p<0.05$). Myeloid lineage cells of BM in the patients gradually increased till the 7th day ($p<0.05$). The proliferative effect of HDMP

on myeloid lineage cells may be due to the increase of serum G-CSF and GM-CSF levels[5]. The serum G-CSF and GM-CSF levels were not evaluated in this study because the increase of serum G-CSF and GM-CSF levels following short-course HDMP treatment have been indicated in patients with ALL previously[5].

We believe that short-course HDMP treatment causes apoptosis on lymphoblasts and proliferation of myeloid lineage cells in children with ALL HDMP-induced apoptosis and the proliferative effect of HDMP on myeloid lineage cells should be evaluated in a larger series of patients with ALL.

REFERENCES

1. Yetgin S, Gürgey A, Tuncer AM, et al. A comparison of the effect of high-dose methylprednisolone with conventional-dose prednisolone in acute lymphoblastic leukemia patients with randomization. Leuk Res 1998; 22: 485-493.
2. Öner AF, Gürgey A, Kirazlı S, et al. Changes of hemostatic factors in children with acute lymphoblastic leukemia receiving combined chemotherapy including high dose methylprednisolone and L-asparaginase. Leuk Lymphoma 1999; 33: 361-364.
3. Hiçsönmez G, Gümrük F, Zamani PV, et al. High-dose methylprednisolone for children with acute lymphoblastic leukemia and unfavorable presenting features. Eur J Hematol 1997; 58: 26-31.
4. Hiçsönmez G, Onat N, Albayrak D, et al. Acceleration of leukocyte recovery by administration of short-course high-dose methylprednisolone in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol 1991; 8: 193-197.
5. Tuncer AM, Hiçsönmez G, Gümrük F, et al. Serum TNF-alfa, gamma-INF, G-CSF and GM-CSF levels in neutropenic children with acute leukemia treated with short-course, high-dose methylprednisolone. Leuk Res 1996; 20: 265-269.
6. Hiçsönmez G, Özsoylu S, Tuncer AM, et al. Direct morphological evidence of high-dose methylprednisolone-induced maturation of leukemic cells in children with acute nonlymphoblastic leukemia. Exp Hematol 1991; 19: 232-233.
7. Hiçsönmez G, Tuncer AM, Çetin M, et al. Morphologic evidence of in vivo differentiation in acute myeloblastic leukemia. Acta Haematol 1993; 90: 214-215.
8. Hiçsönmez G, Erdemli E, Tekelioğlu M, et al. Morphologic evidence of apoptosis in childhood acute myeloblastic leukemia treated with high-dose methylprednisolone. Leuk Lymphoma 1996; 22: 91-96.
9. Hiçsönmez G, Özsoylu S, Tuncer AM. Differentiation of myeloid leukemic cells induced by high-dose methylprednisolone in patients with acute myeloblastic leukemia and its therapeutic potential. Leuk Res 1991; 15: 537-541.
10. Özbek N, Erdemli E, Hiçsönmez G, Okur H, Tekelioğlu M. Effects of methylprednisolone on human myeloid leukemic cell in vitro. Am J Hematol 1999; 60: 255-259.
11. Erduran E, Tekelioğlu Y, Gedik Y, Yıldıran A. Apoptotic effects of heparin on lymphoblasts, neutrophils and mononuclear cells: results of a preliminary in vitro study. Am J Hematol 1999; 61: 90-93.
12. Manaster J, Chezar J, Schurtz-Swirski R, et al. Heparin induces apoptosis in human peripheral blood neutrophils. Br J Hematol 1996; 94: 48-52.
13. Friedmann AM, Weinstein HJ. The role of prognostic features in the treatment of childhood acute lymphoblastic leukemia. The Oncologist 2000; 5: 321-328.
14. Kerr JF, Wyllie AH, Currie AR. Appotosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-252.
15. Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980; 284: 555-556.
16. Arends MJ, Morris RG, Wyllie AH. Apoptosis: the role of endonuclease. Am J Pathol 1990; 136: 593-608.
17. Cohen GM, Sun XM, Snowden RT, et al. Key morphological features of apoptosis may occur in the absence of internucleosomal DNA degradation. Biochem J 1992; 286: 333-334.
18. Darzynkiewics Z, Bruno S, Del Bino G, et al. Features of apoptotic cells measured by flow cytometry. Cytometry 1992; 13: 795-808.
19. Telford WG, King LE, Fraker PJ. Rapid quantitation of apoptosis in pure and heterogeneous cell populations using flow cytometry. J Immunol Methods 1989; 172: 1-16.