Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Application of Interleukin 2 in Neuroblastoma

R. Handgretinger [1], G. Bruchelt [1], M. Schneider [2], D. Niethammer [1], and J. Treuner [1]

A. Introduction

Neuroblastoma is one of the most common solid tumors in childhood, with some unusual neoplastic behavior depending on the age of the affected children. Patients older than 1 year who have metastatic disease have a very poor prognosis. However, in children younger than 1 year who have disseminated disease, regression of neuroblastoma often occurs after only minimal therapy, or even spontaneously. This latter feature and other observations led to long-standing speculations that cytotoxic effector cells of the tumor-bearing host may contribute to this biological behavior of human neuroblastoma [1]. Recently, Main et al. [2] published data concerning the interaction of human neuroblastoma cell lines with effector cells. They demonstrated that the neuroblastoma cell lines CHP 100 and CHP 126 were lysed by natural killer (NK) cells but not by cytotoxic T-lymphocytes [2]. This was interpreted as a consequence of the weak expression of HLA class-I antigens on neuroblastoma cells [3].

In this study we examined whether other neuroblastoma cell lines are susceptible targets for effector cells from healthy individuals and from patients with neuroblastoma. Since Interleukin 2 (IL-2) has considerable stimulating activity on the cytotoxic functions of lymphocytes, we further investigated whether IL-2 can also stimulate the cytotoxic effects of lymphocytes against the neuroblastoma targets. We next addressed the question of whether the concept of the recently described adoptive immunotransfer of lymphokine-activated killer (LAK) cells as an alternative or additional approach to cancer therapy may also be a new therapeutic approach in human neuroblastoma [4]. Since this requires large amounts of cytotoxic lymphocytes, we tried to optimize the conditions for the in vitro generation of activated killer cells using only IL-2.

B. Materials and Methods

I. Isolation and Preincubation of Effector Cells with IL-2

Peripheral mononuclear blood cells (MNBC) from healthy individuals and from children with neuroblastoma before therapy were isolated by Ficoll-Hypaque gradients. Cells were incubated in a 5% CO_2 incubator at 37 °C for 3 days in RPMI 1640 culture medium, supplemented with either 10% heat-inactivated human AB serum or 10% heat-inactivated fetal calf serum (FCS) in the presence or absence of the indicated concentrations of recombinant IL-2 (rIL-2, Biogen).

II. Cultivation of Lymphocytes with rIL-2

For cultivation of lymphocytes, RPMI 1640 culture medium supplemented with 10% AB serum was used. After isolation, lympho-

[1] Universitätskinderklinik, Abtlg. Hämatologie und Onkologie, Rümelinstrasse 19–23, 7400 Tübingen, FRG
[2] Medizinische Universitätsklinik, Otfried Müller Strasse 10, 7400 Tübingen, FRG

cytes were seeded at $3–5 \times 10^5$ cells/ml in the presence of 100 U/ml rIL-2. Fresh culture medium and rIL-2 were added every 3–4 days, and cell concentration was readjusted to 3×10^5 cells/ml.

III. Target Cells

Four established neuroblastoma cell lines with different biological features were used: The well differentiated SK-N-SH, which is dopamine-β-hydroxylase positive and has no N-myc amplification, the only weakly differentiated SK-N-LO, the IMR-5, which has N-myc amplification, and the SK-N-MC, with cholinergic features. For measuring NK activity, the NK-sensitive erythroleukemic cell line K 562 was used.

IV. Proliferation of Lymphocytes

Proliferation of MNBC cultured with different concentrations of rIL-2 for 6 days was quantified with the MTT-proliferation assay [5].

V. Determination of Cell Surface Markers

Lymphocytes were stained with the monoclonal antibodies OKT-3(CD3), OKT-4(CD4), OKT-8 (CD8), Leu-11(CD16), and anti-IL-2 receptor mAb Tü 69(CD25) and were analyzed with a FACS analyzer.

VI. Chromium 51-Release Assay of Cytotoxicity

Briefly, 1×10^6 target cells were labeled with 100 µCi ^{51}Cr for 1 hour, washed, and adjusted to 5×10^4 cells/ml. Aliquots of 0.1 ml were added in triplicates to the effector cells and incubated for 4 hours.

C. Results and Discussion

I. Preincubation of Effector Cells with rIL-2 Stimulates the Cytotoxicity Against Neuroblastoma Targets

MNBC from healthy individuals were preincubated with 100 U/ml rIL-2 for 3 days. Controls consisted of MNBC cultured only in culture medium. Since it is known that FCS may contain some unspecific lymphocyte-stimulating factors, we additionally compared the influence of culturing the MNBC for 3 days in either 10% FCS or 10% AB serum in the absence of rIL-2. There was a significant difference in the spontaneous cytotoxicity of the effector cells against the neuroblastoma targets. MNBC cultured in RPMI 1640–10% FCS consistently became more cytotoxic compared with the effector cells cultured in RPMI 1640–10% AB serum, which showed only modest or no detectable cytotoxicity against the neuroblastoma cells. However, preincubation of the MNBC with 100 U/ml rIL-2 for 3 days resulted in a considerable augmentation of their cytotoxicity against all four neuroblastoma targets. Figure 1 summarizes the results of these experiments. Since preincubation of MNBC in culture medium supplemented with FCS can stimulate the cytotoxicity of lymphocytes through factors present in FCS, and this may therefore mimic an NK sensitivity of target cells, all further experiments were performed in culture medium supplemented with 10% AB serum.

II. Influence of rIL-2 on Effector Cells from Patients with Neuroblastoma

We next investigated whether rIL-2 can also augment the cytotoxicity of lymphocytes isolated from patients with neuroblastoma before therapy. As shown in Table 1, preincubation of MNBC from all patients with rIL-2 for 3 days resulted in a considerable augmentation of the cytotoxicity of the effector cells against the neuroblastoma targets and against the K 562. Unstimulated effector cells showed almost no detectable cytotoxicity against all targets.

III. Proliferation of MNBC in rIL-2

MNBC were incubated with various concentrations of rIL-2 for 6 days. The optimal conditions for inducing proliferation of MNBC were obtained in the presence of 100 U/ml rIL-2 in the culture medium.

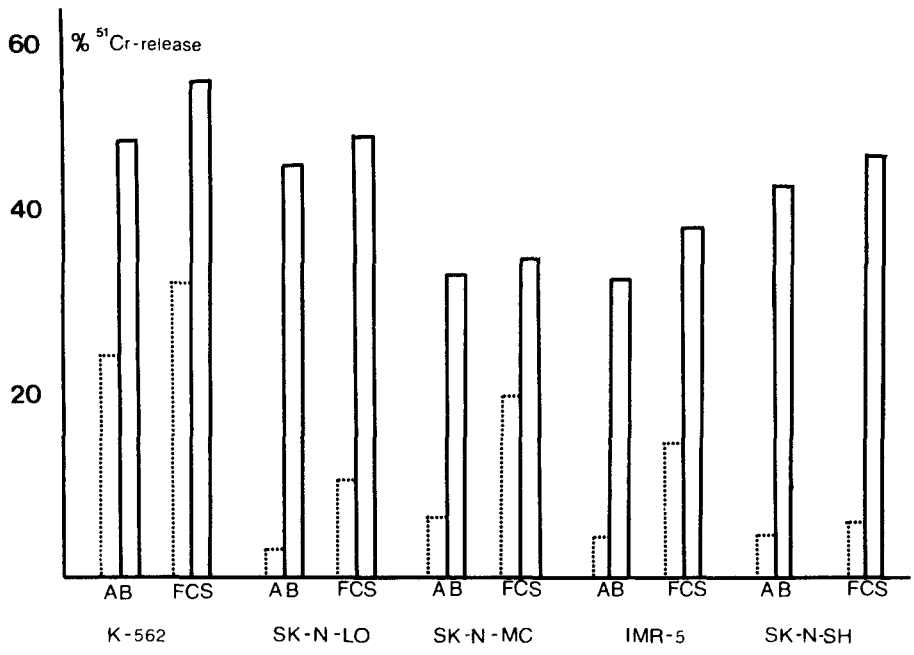

Fig. 1. Susceptibility of different human neuroblastoma cell lines and K 562 as targets for MNBC from healthy individuals preincubated for 3 days with (▭) or without (⬚) IL-2. Culture medium was supplemented with either 10% AB serum (*AB*) or 10% fetal calf serum (*FCS*). E:T-ratio = 10:1

Table 1. Percent of specific ^{51}-Cr-release after 3-day incubation of MNBC with and without IL-2 (E:T = 10:1)

Patient	Target cells							
	SK-N-LO		SK-N-MC		IMR-5		K 562	
	0	100 IL-2	0	100 IL-2	0	100 IL-2	0	100 IL-2
1	0.3%	→54.1%	–		−0.3%	→66.3%	1.4%	→66.6%
2	–		2.2%	→36.2%	–		3.1%	→30.7%
3	1.8%	→30.3%	−1.4%	→18.1%	–		5.8%	→36.7%
4	–		1.1%	→21.9%	–		1.7%	→18.5%
Healthy person	–		0.9%	→26.1%	–		11.2%	→48.3%

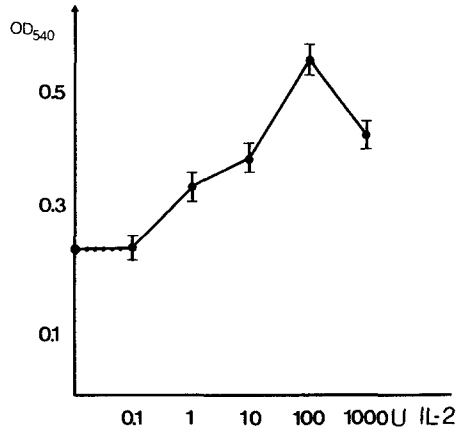

Fig. 2. Proliferation kinetics of MNBC after 6 days' culture with different amounts of IL-2

Higher or lower concentrations of rIL-2 resulted in a lower proliferation rate of lymphocytes, as shown in Fig. 2.

IV. Cultivated Lymphocytes Retain Their Cytotoxic Potential

MNBC from patients with neuroblastoma were cultured in the presence of 100 U/ml rIL-2. Generally, lymphocytes started to proliferate after about 6 days. Starting with 1×10^6 MNBC, about 30×10^6 lymphocytes were obtained after 10–12 days. Cultured lymphocytes were still capable of killing

both the neuroblastoma targets and the K 562 cells to the same degree as after 3 days incubation with 100 U/ml rIL-2. After 12–16 days in continuous culture in the presence of 100 U/ml rIL-2, lymphocytes stopped proliferating and started to disintegrate. However, when the rIL-2 concentration was reduced or omitted for a short period at this point, a following restimulation of MNBC with the original rIL-2 concentration resulted in further proliferation. In this way, prolonged survival of cultured lymphocytes for up to 30 days was possible. Surface marker analysis of the cultured lymphocytes revealed only minimal or no expression of T cell markers or NK cell markers. The phenotype of these cells has still to be determined.

In summary, we showed that the four investigated neuroblastoma cell lines are only weakly susceptible targets for spontaneous cell-mediated cytotoxicity by unstimulated MNBC. Preincubation of MNBC with rIL-2 for 3 days, however, resulted in a strong stimulation of their cytotoxicity against the neuroblastoma targets. Furthermore, it is possible to cultivate MNBC for certain periods of time using rIL-2 alone while retaining their cytotoxic potential. It still has to be tested whether MNBC from patients with neuroblastoma activated with IL-2 are also capable of killing autologous neuroblastoma cells. If this is the case, treatment of these patients with IL-2 and the immunotransfer of lymphokine-activated killer cells could be a new therapeutic approach to neuroblastoma, serving as an adjunct to conventional therapy.

References

1. Bill AH, Morgan A (1970) Evidence for immune reaction to neuroblastoma and future possibilities for investigation. J Pediatr Surg 5:111
2. Main EJ, Lampson LA, Hart MK, Kornbluth J, Wilson DB (1985) Human neuroblastoma cell lines are susceptible to lysis by natural killer cells but not by cytotoxic T lymphocytes. J Immunol 135:242
3. Lampson LA, Fisher CA, Whelan JP (1983) Striking paucity of HLA-A,B,C and β_2-microglobulin on human neuroblastoma cell lines. J Immunol 130:2471
4. Rosenberg SA (1985) Lymphokine-activated killer cells: a new approach to immunotherapy of cancer. JNCI 75:595
5. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55