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NK cell depletion and recovery in SCID mice treated with anti-NK1.1 antibody

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Abstract: The anti-NK1.1 antibody produced by PK136 hybridoma cell line administered subcutaneously to SCID mice effectively decreased the level of peripheral blood NK cells and weight of the spleen for 3-4 days. The antibody treatment did not harm the general state of the animal, and may be practically applied in xenograft experiments. (www.cm-uj.krakow.pl/FHC)

Key words: NK lymphocytes - Anti-NK1.1 antibody - SCID mice

Introduction

The organism of SCID mice (Severe Combined Immunodeficiency) has normal internal microenvironment for hemopoiesis, and develop mature monocytes, polymorphonuclear leukocytes, megakaryocytes, erythrocytes and NK cells. The mice, however, lack both humoral and cell-mediated immunity due to the absence of mature B and T lymphocytes [5]. As the specific immunity of SCID mice is invalid, the organism is used as a convenient model animal for implantation of human normal or pathological cells or organs [14]. Even the mouse with functional human hemopoietic system may be constructed within SCID mouse [4]. The problem is, however, the presence of mouse NK cells attacking the implanted cells.

A convenient method for prolongation of the transplant survival is treatment of the animal with chemotherapeutics (cyclophosphamide, busulfan) [4] or irradiation [8] before xenotransplant implantation. However, the treatment with cytostatics or irradiation, damages not only NK-cells, but also cells of several other tissues and organs, what may influence the result of the experiment. The treatment of mice with specific anti-mouse NK-cells antibody would probably prevent the unwanted host tissue damage. We could not find bibliography concerned with the number of NK cells in the SCID mice after treatment with the anti-NK1.1 antibody. The presented results describe the changes in the number of

NK cells in the peripheral blood and in the spleen of anti-NK1.1 antibody-treated SCID animals.

Materials and methods

Mice. The animals were pathogen-free, our own breeding, 10-12 weeks old severe combined immunodeficient (SCID) mice [5], C.B-17/1crHan-Hsd-*scid*; first families were from Harlan Shaw's Farm, UK. Animals were housed in sterilized microisolator cages and received autoclaved food and drinking water. The mice were systematically controlled for the absence of B and T lymphocytes with the mouse anti-CD19 PE (clone 1D3) and mouse anti-CD3 FITC (clone 17A2), both from Pharmingen. The Local Ethical Committee approved the experiment on animals.

Anti-NK cell antibody. The mice hybridoma cell line (PK136), secreting mouse IgG2a monoclonal antibody reacting with mice NK cells [12] was obtained from the American Type Culture Collection (ATCC). The antibody is specific for NK cells and is cytotoxic in the presence of mouse complement. The SCID C.B-17 mice have high level of complement. The monoclonal antibody reactivity with mouse NK cells was tested cytometrically after IgG2a labeling with the Zenon Alexa Fluor 488 Mouse IgG2a Labeling Kit (lot 75C3-1) from Molecular Probes. Labeling does not require monoclonal antibody pre-purification. The reactivity of PK136 monoclonal antibody is equivalent to anti-NK1.1 antibody [12]. The PK136 cells were maintained in complete RPMI 1640 culture medium supplemented with 10% fetal calf serum (FCS) and antibiotics. The one-day culture medium supernatant collected from several parallel cultures was combined, divided into 1ml portions, and stored frozen at -80°C. The thawed culture medium was used as the source of antibody for experiments. The antibody concentration in the culture medium was estimated by adsorption on protein A-Sepharose column (Pharmacia, lot No 17-0780-01). The eluted IgG fraction was estimated spectrophotometrically by A₂₈₀ absorption assuming absorbance coefficient of IgG=138 [17]. The IgG concentration in the culture medium given to the mouse was 8 µg/ml.

SCID mouse treatment with anti-NK cells antibody. The PK136 culture medium containing anti-NK1.1 antibody was administered

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Table 1. Peripheral blood WBC, number of peripheral blood NK cells, weight of the spleen and number of splenocytes/organ in SCID mice treated with anti-NK1.1 antibody.

	Untreated	Ab treatment 1×			Ab treatment 2×		Ab treatment 3×
		day 2	day 3	day 4	day 1	day 3	day 3
WBC cells/ μ l	825 (550-1125)	800 (500-988)	1000 (888-1188)	650 (613-988)	1000 (888-1053)	900 (600-900)	400 # (400-588)
NK % cells	35 (28-42)	15* (15-19)	14* (13-19)	21 (20-23)	10* (9-11)	13* (12-15)	13* (12-16)
NK cells/ μ l	303 (266-377)	145* (112-175)	208* (175-223)	195 (169-252)	109* (97-117)	142*# (114-147)	86*# (79-139)
spleen weight mg	44.8 (44-51)	43.1 (42-46)	41.1 (37-45)	55.4 (41-50)	37.1* (35-39)	35.7 (29-44)	27.8*# (27-28)
splenocytes/organ $\times 10^6$	29.3 (23-35)	26.7 (23-36)	21.9 (21-28)	23.6 (19-32)	15.1* (15-17)	25.4 (15-25)	15.1* (11-16)

The animals were treated once, 2-times or 3-times and analyzed on the indicated day after the last treatment. Median and percentile values (P25-P75) from n = 3 assays or for untreated control, otherwise n = 4.

p<0.05 as compared with control* or compared with 1× Ab-treated animals#, analyzed on day 3.

subcutaneously once, twice or three times to the animal with 2-day intervals, in the dose of 0.5 ml (4 μ g IgG) per animal. The optimal amount of mAb for *in vivo* NK cell depletion was determined in preliminary experiment by flow cytometry (data not shown), using anti-mouse NK cells antibody (Pan NK Cell PE clone DX5, Pharmingen), in the peripheral blood of treated animals. One, 2, 3 or 4 days after the last injection, the mice were sacrificed and the peripheral blood and spleen were collected. The peripheral blood WBC (white blood cells/ μ l), weight of the spleen and number of splenocytes/organ were determined.

Flow cytometry and statistics. The percentage and number of NK cells/ μ l were analyzed with anti-mouse NK cells antibody (Pan NK Cell PE clone DX5, Pharmingen) and flow cytometry (Becton-Dickinson FACSCalibur) using CellQuest application. The immunocytochemical procedure was as described previously [9]. The median and percentile values were calculated for experiments repeated at least 3 times. The statistically significant difference of results was assumed for p<0.05 in Mann-Whitney U-test.

Results

In peripheral blood of SCID mouse, WBC 825/ μ l was observed and NK cells amounted to 303/ μ l in lymphocyte gate (Table 1). The percent of peripheral blood NK cells was 35% in SCID mice. The absolute values of cell populations (NK cells/ μ l) are more informative than the relative values.

An example of mouse peripheral blood analysis using flow cytometry, 48 h after a double treatment with anti-NK1.1 antibody is presented in Figure 1. The number of lymphocytes per 10 000 leukocytes decreased from 2657 in the control animal (Fig. 1A) to 1045 in the anti-NK1.1 antibody treated mouse (Fig. 1B). Anti-NK-PE positive cells were 3-times lower after treatment (817 cells) than in untreated animal (2357 cells). Two anti-NK1.1 positive cell subpopulations could be observed, one with higher expression of the antibody-recognized domain.

The changes in peripheral blood NK cells depended on the dose of antibody (Table 1). Significantly lower number or NK cells/ μ l was observed after double or triple anti-NK1.1 antibody treatment. The weight of spleen and the number of splenocytes also decreased in 3-times treated animals. However, there was no deterioration of the general state of the treated animals such as lower weight, observed after cyclophosphamide treatment (not presented).

The number of peripheral blood NK cells lowered by anti-NK1.1 antibody treatment rose with time after the last antibody dose (Table 1, 1 × antibody treatment). However 3-4 days after single administration of the antibody, the number of peripheral blood NK cells/l in animal still remained lower by 1/3 as compared with the control, untreated animals.

Discussion

The *scid* mutation was first described in 1983 in CB-17 strain mice [6]. The CB-17 mice are of BALB/c strain with the disrupted gene termed "DNA dependent protein kinase, catalytic subunit" (DNA-PK_{CS}, synonym *Prkdc*) [11,13]. The *Prkdc^{scid}* gene encodes a nonsense mutation which causes the insertion of a termination codon [2]. The lack of an adaptive immune system in SCID mice results from their inability to express rearranged antigen receptors. This is due to a failure to activate a DNA recombinase that requires a functional *Prkdc* gene. As NK cells do not need that enzyme for their function, their activity is undisrupted in CB-17 SCID mice.

CB-17 SCID mice show increased levels of NK cell activity [1]. To eliminate NK cells, SCID mice were treated with anti-asialo GM1 antibody to deplete host NK cells prior to successful implantation of human postnatal thymus [3]. Anti-NK antibody (anti-asialo

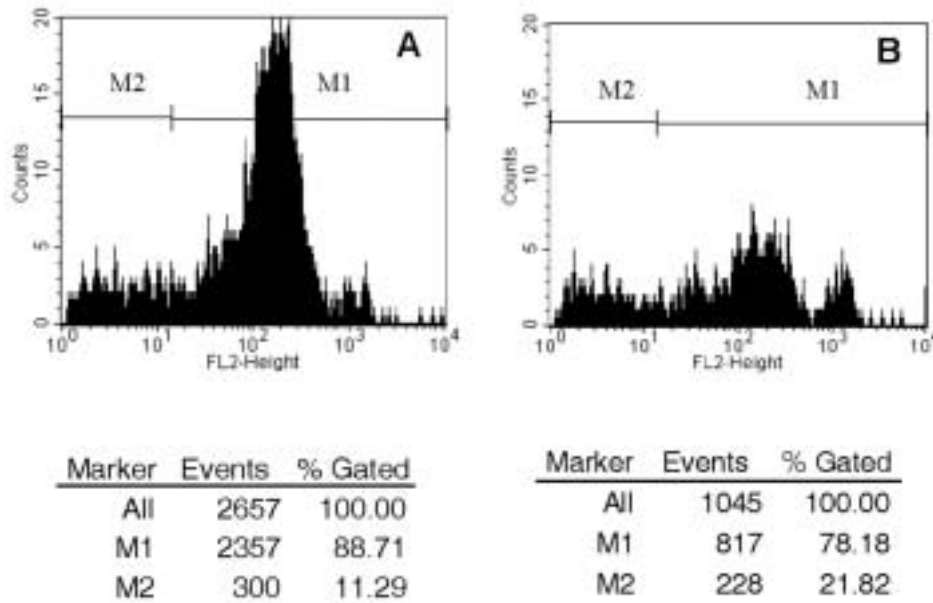


Fig. 1. A representative flow cytometry histograms of peripheral blood lymphocytes labeled with the anti-NK-PE antibody (clone DX5, Pharmingen). Peripheral blood of SCID mouse (A) and SCID mouse treated twice with the anti-NK1.1 antibody (48 h after last treatment) (B). 10000 events were analyzed in the leukocyte gate. M1 - NK-reactive (positive) cells, M2 - NK negative cells.

GM1) in mice enhanced engraftment and chimerism of allogenic fetal liver stem cells as well [7]. In our experiment, however, another anti-NK antibody (anti-NK1.1) was found to decrease the number of NK cells for 3-4 days without any harm to the animal. Probably the continuous treatment with the antibody would prolong the xenograft, *e.g.* human B-chronic lymphocytic cells (B-CLL) survival in SCID mice [10]. Similar experiments on treatment with antisense oligodeoxynucleotides of SCID mice with implanted human K562, *bcr/abl*-positive cell line [15], could be extended in animals treated with anti-NK1.1 antibody.

Apart from the practical application in xenografting experiments in SCID mice, the short period (3-4 days) of NK cell decrease gives a possibility to observe the renewal of these cells. In the model with anti-NK1.1 antibody animal treatment, an information on the role of NK cells *in vivo* may be obtained, *e.g.* in experiments testing the role of NK1.1 cells in protection against MHC Class I⁺ HPV16-associated tumors [16].

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