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NK ACTIVITY OF LYMPHOCYTE SUBSETS AND THE EFFECTS OF LOW DOSE RADIATION*

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Objective: To determine the NK activity of lymphocyte subsets and the effects of low dose radiation. Methods: Lymphocyte subsets were separated by monoclonal antibodies. The NK activity of each subset on tumor cells was detected by radioactive release method. Results: The results showed that besides NK cells, CD_4, CD_8 and B cells alone can kill tumor cells. But the cell-killing activity of NK cells appeared to be strongest. There was synergistic effect between CD_4 and NK cells. The activity of mixed lymphocytes was more than that of only one subset. The effect of low dose radiation (LDR) on NK activity of panlymphocytes or NK cells was different. Conclusion: This paper demonstrated that NK activity of mononuclear cells was called "NK activity of lymphocytes", but it is not true. Only when NK cells were separated by monoclonal antibodies, its killer activity can be called "activity of NK cells".

Key words: NK activity, Monoclonal antibody, Lymphocyte subsets, Low dose radiation.

The natural killer cell (NK cell) which can kill the tumor target cell directly is one of the immune cells. It is different from antibody-dependent killing tumor-cell (K cell). But some researchers regarded all the direct killing tumor-activity of blood lymphocytes as the function of NK cells, so the killing tumor-activity of mononuclear cells separated from peripheral blood lymphocytes represented the activity of NK cells.[1,2] In this paper the main blood lymphocyte subsets were separated from peripheral blood and their killing tumor-activity and regulatory effects were studied. We aim is to study whether the popular method of detecting NK activity reflects the function of NK cells or the killing tumor-activity of panlymphocytes.

MATERIALS AND METHODS

Separation of Lymphocyte Subsets with Panning-direct Method

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood samples by centrifugation over a Ficoll-Hypaque (Shanghai Rongsheng Biological Chemical Factory, density:1.077± 0.002) density gradient. Mononuclear cells (MNCs) and MNCs of non-monocytes were separated by the Petri dishes with medium 1640.[3] McAb CD_4, CD_8 (Academy of Military Medical Sciences), McAb CD_{19} (Sigma Company) and McAb CD_{57} (Huamei Biological Technique Company) were used to separate CD_4, CD_8, CD_{19} and CD_{57} subset cells with panning-direct method established by Wysocki.[4]

Separation of Lymphocyte Subsets with Indirect Method

The obtained MNCs of non-monocytes were first combined with each McAb separately and then incubated with goat-anti-mouse IgG to get CD_4, CD_8,

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CD_{19} subset cells and non-CD_4, non-CD_8 and non-CD_{19} cells.

Detection of Purity, Survival and Yield of Lymphocyte Subsets

The lymphocyte subsets obtained from two kinds of methods were detected by reported method.[3] The McAb positive cells mixed with non-CD_4, non-CD_8, or non-CD_{19} cells were also detected by the same method.

Irradiation Condition

Ra-226 gamma rays source (radioactivity: 3.89×10^7 Bq) was put on the bottom of incubator. The cell cultures were put on the plank which was 25 cm above the Ra-226 source. The dose rate of irradiation was 1.3 cGy/h.

Target Cell

K_{562} cells were used as target cells that were cultured in RPMI 1640 medium contained 10% FCS. When the cells were at logarithm proliferation stage, 3H-TdR 3.7×10^4 Bq was added into every culture medium and kept for 6 h at 37℃. The cells were washed three times with PBS.

Determination of Activity of Killing Tumor-cell

Labelled target cells in amount of 1×10^5 and effector cells (CD_4, CD_8, CD_{19}, CD_{57}) were put in bottles which contained 3 ml RPMI 1640 culture medium with AB blood serum. The ratio of effector : target cells was 5:1. When cooperation between two lymphocyte subsets was studied the number of every subset per bottle was half of the total. At the same time single target cells group and control group of effector cells were made. These samples were incubated for 16 h at 37℃ and 0.15% pancreas enzyme and 0.0125% deoxyribonuclease were added for 30 min. The cells were collected on glass fiber discs[4] and residual radioactivity in tumor cells was measured in CPM. The NK activity was shown as follows:

NK activity=CPM of target cell group-CPM of (effector +target) cell group

Ratio of NK activity of irradiated group: non-irradiated group=CPM of target cell group-CPM of irradiated group/CPM of target cell group-CPM of control group

RESULTS

Purity, Survival and Yield of Lymphocyte Subsets Separated by McAbs

The purity and survival of subset cells separated by panning-direct method was high but the yield was a little low, just the same as reported result.[5] The purity and survival of four kinds of lymphocyte subsets were beneficial to experiment (Table 1).

The purity of CD_4, CD_8 and CD_{19} cells separated by indirect method were 90%, 91% and 82% respectively. There is 6% CD_4 cells in non-CD_4 cell group and 5% CD_8 cells in non-CD_8 group, 9% CD_{19} cells in non-CD_{19} group. The survival of each subset cells and non-adherent cells was over 92%.

Table 1. Purity, survival and yield of lymphocyte subsets separated by McAbs (%, $\bar{x}\pm s$)

Index	CD_4	CD_8	CD_{19} (B)	CD_{57} (NK)
Purity	89.3± 3.5	91.3± 1.5	91.5± 1.1	90.5± 3.4
Survival	96.0± 1.9	95.8± 1.5	96.0± 2.4	95.3± 1.5
Yield	26.5± 4.4	18.7± 2.3	16.3± 1.9	14.8± 2.4

n=4

The Activity of Killing Tumor by Lymphocyte Subset and the Synergistic Effects of CD_4, CD_8, CD_{19} Cells on CD_{57} Cells

The activity of killing tumor by each subset cells separated by panning-direct method is shown in Table 2.

The results showed that the NK activity was found in all of the four subsets. But the NK activity of CD_4 or CD_{19} cells was lower than that of CD_{57} (NK)

cells significantly.

Table 2. NK activity of lymphocyte subsets and the regulatory effects between them ($\bar{x}\pm s$)

Group	NK activity (CPM)	Compared group
1. CD_4	941± 203	1:3**
2. CD_{19}	921± 214	2:3**
3. CD_{57}	1582± 325	
4. CD_4+CD_{57}	1779± 372	4:5*
5. CD_{19}+CD_{57}	1187± 289	4:6**
6. CD_8+CD_{57}	1079± 251	(1+3) : 4 (×2)*

n=5 *P<0.05, **P<0.01

The NK activity of CD_4+CD_{57} group was stronger than that of CD_{19}+CD_{57} group or CD_8+CD_{57} group. The sum of the CPM of two single subsets was compared with two times of the CPM of co-cultured group when regulatory effect was analysed (number of effector cells is equal). The result showed that when CD_4 and CD_{57} cells were co-cultured, the total NK activity was higher than that of the sum of two single subsets incubated separately, which demonstrated that there was synergistic effect between CD_4 and CD_{57} cells. When CD_{19} cells co-cultured with CD_{57} cells, no synergistic or suppressive effect was found.

Killing Tumor-activity of Each Lymphocyte Subsets and Their Replenished Cell Group

The activity of killing tumor by each lymphocyte subsets separated by indirect method is shown in Table 3. The killing activity of each replenished cell group was higher than that of only one subset. No significant difference was found among all subsets or among all replenished cell groups.

The Effects of LDR on the NK Activity of Blood Lymphocytes and CD_{57} Cells

Mononuclear cells of normal subjects or CD_{57} cells which has been irradiated with gamma rays were co-cultured with tumor target cells. The ratio of NK activity of irradiated group to non-irradiated group is shown in Table 4. When mononuclear cells were irradiated with 10 cGy gamma rays the NK activity began to increase. The increase of NK activity was greatest after 50 cGy gamma rays irradiation. As to CD_{57} cells its NK activity began to increase significantly after 50 cGy gamma rays radiation and when the radiation dose was 80 cGy, there was still stimulating effect on CD_{57} cell.

Table 3. NK activity of three subsets and their corresponding replenished cell groups ($\bar{x}\pm s$)

Group	NK activity (CPM)	Group	NK activity (CPM)	P
CD_4	1375±305	Non-CD_4	2045±596	<0.05
CD_8	1115±530	Non-CD_8	2080±994	<0.05
CD_{19}	1185±305	Non-CD_{19}	2100±1087	<0.05

n=7

Table 4. Effects of LDR on NK activity (ratio of NK activity of irradiated group to non-irradiated group $\bar{x}\pm s$)

Dose (cGy)	n	Total lymphocytes	n	CD_{57} cells
0	10	100	6	100
10	10	120.8± 21.0*	6	102.7± 6.7
30	– –	– – –	6	106.2± 7.6
50	10	140.0± 21.4**	6	128.5± 8.7**
80	10	108.4± 17.5	6	134.2± 12.3**
120	8	101.7± 5.2	6	102.3± 10.1

Compared with 0 cGy group *P<0.05, **P<0.01

DISCUSSION

In this study four kinds of lymphocyte subsets were separated. Besides NK cells, CD_4, CD_8 and CD_{19} cells can also kill tumor target cells. Among them, CD_{57} cell appeared to be of the strongest killing activity, higher than that of CD_4 or CD_{19} cell. There was synergistic effect between CD_4 and CD_{57} cells. The killing activity of CD_4+NK cell group was significantly higher than that of CD_8+CD_{57} group or CD_{19}+CD_{57} group. Another experiment also showed that killing activity of group CD_4+NK cell or non-CD_{19}+NK cell was higher than that of group CD_8+NK cell or group CD_{19}+NK cell. In general CD_4 cell belongs to T-helping lymphocyte and the majority of non-CD_{19} cells is T cells. Our results indicated that most cells of killing tumor are T cell.[6] Table 3 showed that killing activity of a single subset was not so strong as that of its corresponding replenished cell group. It indicates the importance of synergistic joint action among immune cells in tumor immunity.

LDR can stimulate the function of several kinds of immune cells.[7] In this study when blood lymphocytes were irradiated with 10 cGy gamma rays, its NK activity increased significantly and the NK activity of NK cells (CD_{57} cell) didn't increase until the irradiation dose got to 50 cGy. When blood lymphocyte was given 80 cGy gamma rays irradiation it appeared no stimulating effect but NK cell appeared the strongest stimulating effect. Our experiment before[7] also suggested that 10 cGy irradiation can stimulate the function of T, B lymphocyte. NK cell is a relative radiation resistant cell line. Our results indicated this phenomenon.

This study suggested that the NK activity of total lymphocytes and killing activity of NK cells is different.

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