Short Communication

Natural Killer Cell Activity in Mice after Intraperitoneal Administration of Beryllium Chloride

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Natural killer (NK) cells are cytotoxic lymphocytes that are present in the blood, spleen, lymph nodes and abdominal cavity of humans and animals. There are asialo GM1 antigens¹⁾ as well as many other antigens on the surfaces of their membranes, particularly in mice. As NK cells are supposed to play an important role in protecting the living body against the development of tumors and viral infections, they are regarded as one of the immunological parameters for the defense mechanism of the body^{2–4)}. Beryllium (Be) is reported to be carcinogenic to humans and animals⁵⁾. The carcinogenic mechanisms of Be, however, have not yet been studied in relation to NK cell activities. The aim of this study is to examine the influence of Be on NK cells to seek a clue to the carcinogenic mechanisms of Be.

Material and Methods

Experimental animals and Beryllium solution

Thirty male C57BL/6CrSle mice (5 wk of age, Charles River Japan, Kanagawa, Japan) were used. The mice were divided into three groups, each of ten animals: two Be injected groups and one control group. In the two Be injected groups, beryllium chloride was intraperitoneally injected into mice at a dose of 1/200 (Ex-1) or 1/100 (Ex-2) of the LD₅₀ (12 mg/kg)⁶⁾ for one intraperitoneal injection, once every other day, 5 times altogether. In the control group, physiological saline having the same pH (pH 4.1) as beryllium chloride solution was given by the same procedure as in the Be groups. Beryllium solution was prepared by dissolving beryllium chloride (BeCl₂, Mitsuwa Chemicals, Osaka, Japan) in physiological saline solution and sterilized through a

millipore filter (Millex GV, 0.22 µm, Millipore Japan, Ltd., Tokyo, Japan).

Preparation of target and effector cells

Target cells were prepared by the method of Nunoyama *et al*⁷⁾. YAC-1 cells (Dainippon Pharmaceutical Co., Ltd, Osaka, Japan) derived from mouse lymphoma were used as target cells. The target cells (1.0 ml solution, 1 × 10⁷ cells/ml) were labeled with 0.1 ml of 37 MBq sodium chromate in 0.9% sodium chloride solution (Chromium-51, Amersham Life Science, Tokyo, Japan) at 37°C for 45 min. Then, the cells were washed in RPMI-1640 medium (RPMI1640, GIBCO Life Technologies, New York, USA) containing 10% heat-inactivated fetal calf serum (FCS, GIBCO), adjusted to a concentration of 2 × 10⁵ cells/ml in RPMI-1640 medium containing 10% heat-inactivated FCS. Spleen cells of the mice were used as effector cells. Spleen cells of the mice were prepared by the method of Yahara and Edelman⁸⁾ and the concentration of cell suspensions was adjusted to 1 × 10⁷ cells/ml.

Measurement of NK cell activity and the proportion of asialo GM1⁺ cells

NK cell activity was measured in round-bottom 96-well microtiter plates in triplicate according to the method of Nunoyama *et al*⁷⁾. Effector cells were mixed with target cells (2 × 10⁵ per well) at an effector-target cell ratio of 50:1. After 5 h of incubation (37°C, 5% CO₂), the supernatant was collected from the wells by means of a supernatant collection system (Skatron, Norway). The release of radioactivity from target cells was determined with a gamma counter (Crystal II, model B5424, Packard Instrument, Co., USA). Then, NK cell activity was calculated by the following formula:

NK cell activity (%) = {(E–S) / (M–S)} × 100, where E is the experimental release of radioactivity from target cells induced by effector cells. S is the spontaneous release of radioactivity from target cells alone. M is the maximum release of radioactivity from target cells caused by 10% Triton X-100. NK cell activity per NK cell was estimated by dividing NK cell activity by the proportion of asialo GM1⁺ cells. According to the method of Nakamura *et al*⁹⁾, spleen cells were incubated at 4°C with anti-asialo GM1 antibody (Anti Asialo GM1, Rabbit, Wako Pure Chemical Industries, Osaka, Japan) for 30 min. After being washed with phosphate buffer saline (PBS), the cells were incubated at 4°C with anti-rabbit FITC IgG (Dako Japan, Co., Ltd, Kyoto, Japan) for 30 min. Then, after rewashing, the cells were centrifuged at 800 rpm for 10 min, and the cell pellets were suspended in PBS containing 1% formalin. The asialo GM1⁺ cells of the cell pellets suspending in PBS were analyzed with a fluorescence activated cell sorter (FACS, Becton Dickinson, New Jersey, USA). The proportion of asialo GM1⁺ cells to two thousand spleen cells was calculated.

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Fig. 1. Natural killer (NK) cell activity in mice intraperitoneally injected with beryllium chloride. The mean values for NK cell activity are presented with group means $\pm$ SD (vertical bars). 1/200 of the LD₅₀ (Ex-1): group treated with 1/200 of the LD₅₀ for intraperitoneal injection, 1/100 of the LD₅₀ (Ex-2): group treated with 1/100 of the LD₅₀. *** p <0.001 (One-way ANOVA and post hoc multiple comparisons by means of Dunnett's pairwise multiple comparison t test).

Statistical methods

One-way ANOVA and post hoc multiple comparisons by means of Dunnett's pairwise multiple comparison t test were applied for testing the effect of Be administration. The Pearson's correlation coefficient was calculated to test the association between NK cell activity and the proportion of GM1⁺ cells in a pooled group in which Ex-1, Ex-2 and control groups were combined.

Results

NK cell activity was significantly lower in the Ex-2 group (p <0.001) than in the control group (Fig. 1). NK cell activity tended to decrease dose-dependently. The mean proportion of asialo GM1⁺ cells in the Ex-2 group was significantly lower than that in the control group (p =0.011) (Fig. 2). A good correlation was found between NK cell activity and the proportion of asialo GM1⁺ cells in a pooled group (r =0.531, p =0.003). The NK cell activity per NK cell was also significantly lower in the Ex-1 group (p =0.008) and Ex-2 group (p =0.002) than that in the control group (Fig. 3).

Discussion

In the present study we found that the NK cell activity and that per NK cell were lower in the Be administration groups than those in the control group. The proportion of asialo GM1⁺ cells to the spleen cells was also lower in one group with the administration of a higher Be dose. A significant correlation was found between the NK cell

Fig. 2. Proportion of asialo GM1⁺ cells to spleen cells in mice intraperitoneally injected with beryllium chloride. The mean proportions are presented with group means $\pm$ SD (vertical bars). 1/200 of the LD₅₀ (Ex-1): group treated with 1/200 of the LD₅₀ for intraperitoneal injection, 1/100 of the LD₅₀ (Ex-2): group treated with 1/100 of the LD₅₀. * p =0.011 (One-way ANOVA and post hoc multiple comparisons by means of Dunnett's pairwise multiple comparison t test).

Fig. 3. Natural killer (NK) cell activity per NK cell in mice intraperitoneally injected with beryllium chloride. The mean values for NK cell activity are presented with group means $\pm$ SD (vertical bars). 1/200 of the LD₅₀ (Ex-1): group treated with 1/200 of the LD₅₀ for intraperitoneal injection, 1/100 of the LD₅₀ (Ex-2): group treated with 1/100 of the LD₅₀. ** p =0.008, *** p =0.002 (One-way ANOVA and post hoc multiple comparisons by means of Dunnett's pairwise multiple comparison t test).

activity and the proportion of asialo GM1⁺ cells in spleen cells. This finding suggests that the decrease in NK cell activity in the groups with Be administration is due to a decrease in the proportion of asialo GM1⁺ cells in spleen cells. The asialo GM1 antigen is one of the antigens on the surface of the NK cell membrane¹⁾. It is therefore

suggested that the decrease in the NK cell activity is caused by a decrease in the proportion of NK cells to spleen cells, but the decrease in NK cell activity might be simply explained by the lowering of NK cell activity per NK cell, even when the proportion of NK cells to spleen cells is unchanged.

Helper T (T_H) cells have a relationship to the differentiation and proliferation of NK cells^{10, 11)}. There is a study reporting a decrease in the proportion of L3T4⁺ cells and an increase in the proportion of Ia⁺ cells to spleen cells in Be administered mice¹²⁾. L3T4 antigen is known to exist on the surface of the mouse T_H cell membrane. Ia antigen is also reported to be present on the surfaces of the membranes of B cells and antigen presenting cells in mice. It is therefore necessary to study whether the decrease in the proportion of asialo GM1⁺ cells to spleen cells in the present study is related to a decrease in the proportion of L3T4⁺ cells, or an increase in the proportion of Ia⁺ cells to spleen cells, or both.

There are studies which reported a decrease in NK cell activity per NK cell and an increase in the proportion of NK cells to mononuclear cells in the peripheral blood of workers exposed to occupational carcinogens^{13, 14)}. The decrease in NK cell activity per NK cell is consistent with our result on Be, but the increase in the proportion of NK cells among workers exposed to carcinogens would be inconsistent with the decrease in the proportion of NK cells suspected in our study. As the reason for this difference between the effects of carcinogens and Be is unknown, further study is needed on their toxicological mechanisms. It is also necessary to study whether the lowering of NK cell activity found in the present animal occurs in workers exposed to Be in the workplace.

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