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Brief Note

Natural Killer Cell Activity and Complement Activity in Benzene-intoxicated Rats

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Key words : Natural killer cell — Complement — Benzene intoxication

Natural killer cells (NK cells) are specialized lymphocytes of bone marrow origin that may play important roles in the surveillance of certain tumors and virusinfected cells.[1—3] It is also well known that benzene is regarded as a hazardous hydrocarbon principally because of its myelotoxicity and leukemogenesis.[4]

In this study, we examine the effects of successive subcutaneous administrations of benzene on NK cell activity in peripheral blood lymphocytes and on plasma complement levels in rats.

MATERIALS AND METHODS

Animals : Male Wistar rats, 9 weeks old, were used in this study. Animals were fed Oriental MF pellets and given tap water *ad libitum*. Two groups of 5 rats each were treated as follows: one group was given 10 subcutaneous injections of benzene dissolved in sterilized olive oil (1 : 1 v/v) daily at a dosage of 2.0 ml/kg of body weight during a period of 12 days. The other group received 2 ml/kg of olive oil in an identical manner and served as a control. Animals were sacrificed 24 hours after the final injection, and blood was drawn from the aorta with heparinized plastic syringes. Then plasma was separated by centrifugation at 4°C.

Target cells : YAC-1 tumor cells (kindly provided by Professor K. Ito, Tohoku University Dental School) were maintained *in vitro* in RPMI 1640 medium (Gibco, N. Y.) supplemented with 10 percent fetal calf serum (FCS).

Effector cells : Large granular lymphocytes were separated from 2 ml of blood after dilution with an equal volume of phosphate buffer saline, by a Ficol-sodium metrizoate density gradient centrifugation method. NK cells, purified by a Percoll density centrifugation method, are shown in Figure. 1.

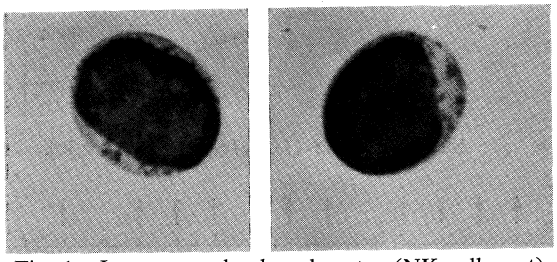

Fig. 1. Large granular lymphocytes (NK cells, rat) (Wright stain, ×2600).

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Cytotoxic assay : NK-cell activity in peripheral blood was examined *in vitro* by a modification of the ^{51}Cr release assay described by Kiessling *et al.*[5] ^{51}Cr labeled YAC-1 cells were used as target cells. 7.5×10^6 target cells in 0.6 ml of RPMI 1640 medium (10 percent CFS) were labeled with 500 μCi of ^{51}Cr ($Na_2{}^{51}CrO_4$) in 0.5 ml saline for 1 hour at 37°C, washed twice with the medium, let stand at 4°C in a refrigerator for 30 minutes, then washed again with the medium and resuspended in RPMI 1640 medium (10 percent FCS) at a concentration of 2×10^6 cells/ml. ^{51}Cr-labeled target cells(4×10^4 in 40 μl) were cocultured in quadruplicate with effector cells (1.6×10^6 in 150 μl) in round-bottomed microtiter plates at an effector : target ratio of 40 : 1, while control wells contained target cells only. The microplates were incubated at 37°C in a 5 percent CO_2 atmosphere for 4 hours. After the incubation, cell free supernatant from each well was collected by centrifugation and ^{51}Cr activity was counted in a well type scintillation counter. The specific ^{51}Cr release was determined by calculating the ratio of ^{51}Cr activity in the supernatant and ^{51}Cr activity in total added target cells.

Complement activity assay : The plasma hemolytic complement titer in terms of 50 percent hemolysis (CH50) was assayed by a modified method of Mayer.[6] The plasma C3 level was determined by a single radial immunodiffusion technique (SRID).[7] Total protein concentration in plasma was measured by a modified biuret method (Wako).

Plasma GOT and GPT assays : Glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in plasma were assayed by a commercial kit based on the method of Reitman and Frankel[8] (Transaminase B-Test, Wako).

Hematological examinations : Leukocytes and erythrocyte counts in peripheral blood were determined by a Coulter-Counter (Type Z) and the classification of leukocytes was done by a routine blood smear method.

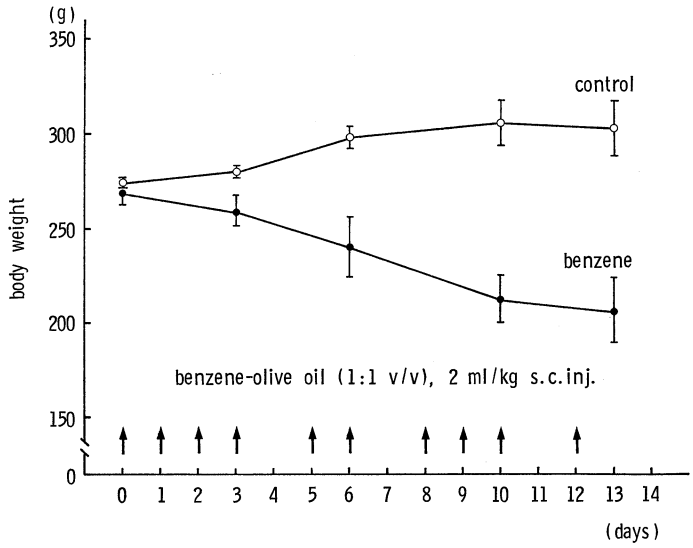

Fig. 2. Effect of benzene on body weight.

Histopathological examination : The livers, spleens, thymuses and femoral bone marrows were immediately removed at dissection, cleaned, weighed and small portions of each organ were fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for histologic examination.

RESULTS AND DISCUSSION

During the course of benzene treatment, animals rapidly lost weight, and at the end of treatment, the mean body weight was about two thirds that of controls (Fig. 2). Weights of the spleens and thymuses per 100 g of body weight in the benzene-treated rats were significantly less than those of controls (Table 1).

The mean percentage of NK cell activity in peripheral blood lymphocytes from the benzene-treated rats was significantly lower than in those from controls. In the peripheral blood of benzene-treated rats, a leukopenia with relative neutrophilia, lymphocytopenia and monocytopenia was evident, while the erythrocyte count was not affected. Mean levels of hemolytic complement activity and C3 concentration in the plasma of the benzene-treated rats were slightly lower than controls, and the results are supported by a finding of a decreased serum complement level in workers exposed to benzene, toluene and xylene.[9]

Histopathological observations of the bone marrows, spleens and thymuses of the benzene treated rats and control rats revealed that the hemopoietic activity of these organs was evidently impaired by benzene treatment (Figs. 3, 4). These findings are similar to those described in other literature.[4,10,11]

In summary, the findings of the present investigation demonstrate that succesive subcutaneous administrations of benzene in rats result in significant suppression of NK cell activity in peripheral blood lymphocytes and plasma complement activity, and it seems reasonable to suggest that benzene exposure may alter to some extent the natural host defense mechanism against infection and carcinogenesis.

TABLE 1. Effects of benzene on NK activity and hemopoietic organs.

Treatment	No. of rats	PBL[a] % ^{51}Cr release	WBC/mm^3	RBC $\times 10^4$/mm^3	Spleen g/100g b.w.	Thymus mg/100g b.w.	Body weight g
olive oil	5	16.61±2.65	8265±547	640±29	0.28±0.01	133±2	302±6
benzene	4	7.07±1.47**	4398±220**	649±16	0.14±0.01**	29±3**	206±9**

mean ± s.d. a) peripheral blood lymphocytes

TABLE 2. Effects of benzene on plasma complement activity and liver function.

Treatment	No. of rats	CH50	C_3 %	Total Protein g/dl	GOT KU/ml[b]	GPT KU/ml	Liver g/100g b.w.
olive oil	5	93.2±7.9	100±2.3	5.5±0.04	146±10	37±5	3.7±0.1
benzene	4	80.3±7.5	82.1±4.3*	5.3±0.10	114±17	23±4	4.2±0.3

significantly below the control ; *$p<0.05$ **$p<0.01$ b) Karmen Unit

Part of this study was presented at the 53rd Annual Meeting of the Japanese Society for Hygiene, Osaka, Japan, April 7, 1983.

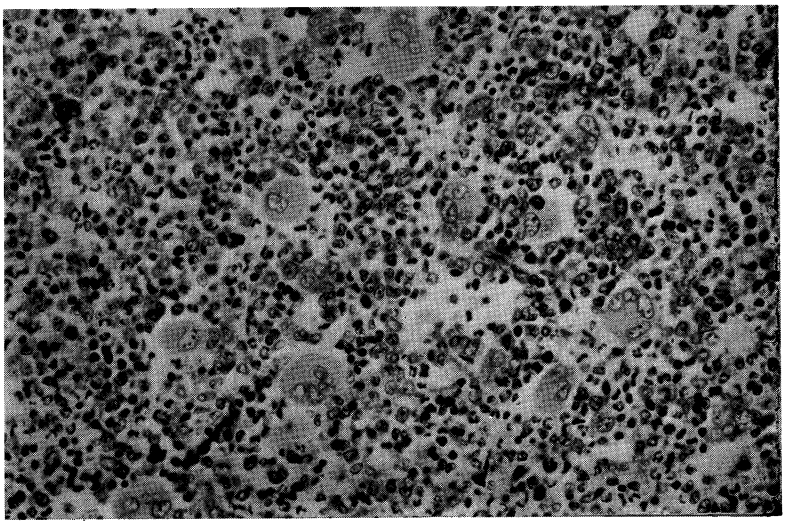

Fig. 3. Femoral bone marrow (control rat) (H.E. stain, ×280).

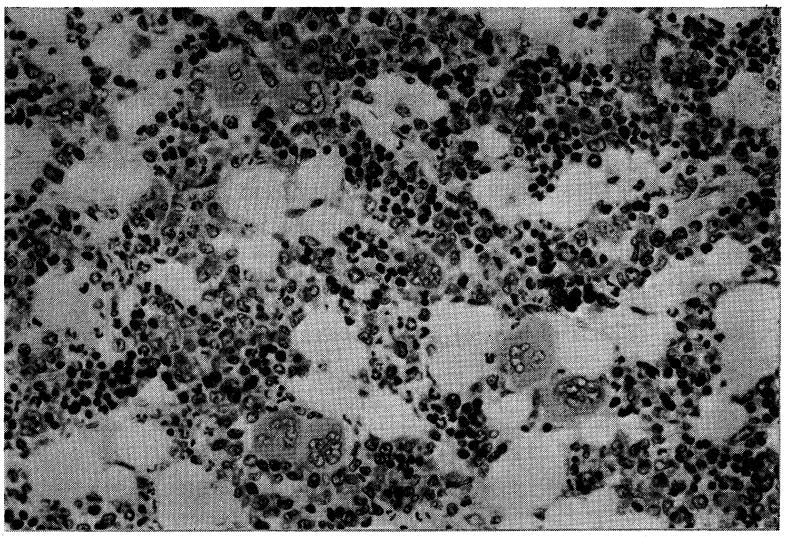

Fig. 4. Femoral bone marrow (benzene-treated rat) (H.E. stain, ×280).

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