ABSTRACT. In an attempt to study the immunological effects on normal and adenocarcinoma (AC) dogs, the natural killer (NK) activity was determined. Augmentation of NK cell activities in responsive normal and AC dogs that indicated anergy in the phaseolus vulgaris agglutinin (PHA) skin-test was manifested when the animals were accordingly treated with germanium (GN).

NOTE Immunology

Effect of Germanium, Poly-trans-[2-Carboxyethyl] Germasesquioxane on Natural Killer (NK) Activity in Dogs

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The prevalence of a protein that suppresses cellular immunosuppression in serum of tumor-bearing dogs that manifest immunohypofunction has been demonstrated [6]. However, potentiation of cell immunity, especially in canines with poly-trans-[2-carboxyethyl] germanium (GN)-enhanced natural killer (NK) activity, has not been reported in Japan. According to Asou et al. [1], interferon (IFN) is induced to subsequently enhance NK cell [3] and macrophage (Mø) activities when GN is orally administered in mice, indicating that GN may serve as a useful (modifying) agent of the immunosystem [5, 8].

As such, we administered GN to normal and AC dogs in the present study to investigate if NK cell activity was enhanced with GN treatment.

Five male healthy Beagles and 1 case diagnosed with AC were orally administered with GN (50 mg/kg: Asai germanium, Tokyo) at 2 administrations/day on alternate days for 2 weeks.

Blood sampling was conducted 4 hr after the last administration. Moreover normal and AC hosts’ whole peripheral blood lymphocytes (WPBL), prepared by Ficoll-metrizoate (lymphoprep; Nyegaard, Norway) after peripheral blood extraction, were washed with RPMI-1640 medium containing 5% FCS. WPBL isolated from peripheral blood were extracted, were washed with RPMI-1640 medium containing 5% FCS. WPBL isolated from peripheral blood were then categorized into non-adherent cells as NK-rich lymphocytic fraction and adherent cells as Mø fraction within 24 hr, and employed as the effector cells thereafter.

NK activity was monitored with 51Cr-labelled canine lymphocytic leukemia (CL-1) as the target cells. WPBL-derived non-adherent NK-rich lymphocytic cells and adherent cells were co-cultured with and without 51Cr-labeled CL-1 cell lines at 37°C in an atmosphere with 5% CO2 for 4 hr. The effector to target cell (E/T) ratios were 25, 50 and 100 to 1 (×104 cells). The percentage of NK activity was calculated according to following equation:

\[
\text{NK activity} (\%) = \frac{\text{[c.p.m. of maximum 51Cr-release} - \text{c.p.m. of spontaneous 51Cr-release}] - \text{[c.p.m. of experimental 51Cr-release} - \text{c.p.m. of spontaneous 51Cr-release}]}{\times 100} 
\]

Radioactivity of 51Cr-released from target cells was counted by a multicrystal gamma counter (LB2101, Belthold, Germany).

In addition, phaseolus vulgaris agglutinin (PHA) skin-test (10 µg/ml: Wako Pure Chemicals Co., Ltd., Japan) was performed as intracutaneous reaction with PHA 12 hr before the first administration and 6 hr after the last administration. In each skin-test, the maximum diameter of erythema induced at 24 hr post-injection was measured with calipers.

\[\text{NK activity on effector cells in normal control dogs: WPBL-derived adherent and non-adherent cells as effector cells isolated 24 hr after blood sampling were used. WPBL samples were taken from the 5 normal dogs. When the NK activity of effector cells, either adherent or non-adherent cells, was measured, the latter manifested a higher cytotoxicity to non-adherent cells (p<0.05 vs. non-adherent cells by method of Wilcoxon), although the NK activity of individual dogs showed slight differences (Table 1).}\]

Augmented effects of GN on NK activity against non-adherent cells in normal dogs: Of the 5 normal dogs used, 2 cases were administered with GN while the remaining 3 cases were treated with the vehicle (saline). Based on the mean of 3 measured values, the cytotoxicity was plotted against the E/T ratio, and the NK activity of the GN-treated group was enhanced more than that of vehicle-treated dogs (Fig. 1). No apparent abnormal or adverse effects were produced with oral administration of GN.

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Table 1. Canine natural killer (NK) activities (%) of target cells separately co-cultured with their respective effector cells for 24 hr at 37°C before assay. Whole peripheral blood lymphocytes (WPBL) were comprised of non-adherent and adherent cells

<table>
<thead>
<tr>
<th>Effector Cells</th>
<th>% of NK activity at E/T ratio (%)</th>
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<tbody>
<tr>
<td></td>
<td>25:1</td>
</tr>
<tr>
<td>WPBL</td>
<td>NDa</td>
</tr>
<tr>
<td>Non-adherent cells</td>
<td>10.9 ± 2.1</td>
</tr>
<tr>
<td>Adherent cells</td>
<td>17.0 ± 2.3</td>
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a) Mean ± SD (%), b) Not done. *: p<0.05 vs. non-adherent cells.
adherent cells in the AC dog: The affected dog (T,N,M) indicated loss of appetite and suffered from nasal bleeding for 2 years before it was diagnosed in our hospital. Swelling was manifested at the mid portion of the dorsal nasal bone, and it was diagnosed to suffer from AC with concurrent radiographic findings revealing bone resorption at the affected site. As results demonstrated that the NK activity of normal dogs was augmented with oral GN administration, GN was thus administered in the AC dog to evaluate the therapeutic potential of this agent, and the NK activities before and after GN treatment were compared (Fig. 2). The AC dog that showed anergy to PHA skin-test indicated negligible NK activities before GN treatment, and the immunosuppressive tendency was parallel to the PHA skin-test, although the intracutaneous reaction in normal dogs registered 1.6 ± 0.5 cm (mean ± S.D.). After administering GN orally alone to the normal and AC dogs over a period of 2 weeks, the NK activity in the latter elevated to 6.5%, corresponding to a value approximating to slightly less than 2 fold that of non-treated normal dogs (p<0.05 vs. without GN by method of Wilcoxon). GN elicited enhancement in NK activities of both normal and AC dogs, and the increase in NK activity (relative to the baseline activity without treatment) was more effective and potent in the latter.

The cytotoxic effect of NK cells [3] against tumor cells of lymphocytes was obviously enhanced in the GN-treated dog, although IFN production and time-related changes in NK activity were not monitored in the present study. As such, studies on immunity-related events after GN treatment are warranted. In mice, i.p. administrations of GN activate peritoneal Mø and promote its cytotoxic effects in cultured tumor cells [2, 7]. In our preliminary studies, the cytotoxic effects were promoted when GN was added to the supernatant of cultured immunity-related cells such as Mø, implying that the production of cytokine as IFN etc. was involved.

Augmentation of cell immunity by gamma-interferon (IFN-γ) production evoked by GN administration [1] has rendered rats and mice to elicit anti-tumor effects [5, 8]. The results of our present study on the clinical effects of GN on responsive normal and AC dogs indicating anergy to PHA skin-test revealed that the NK activities in both normal and AC dogs were augmented (Figs. 1, 2). The cytotoxic effect of NK cells renders the NK cells to recognize non-self cells and therefore proceeds to attack these cells, contributing in short to the anti-tumor effects of the immunosystem [4].

Although NK activities were initially not established in the AC dog, continuous GN administrations over a 2-week period yielded enhancement in the NK activity of WPBL. This finding advocates that augmentation in immunity was promoted by production of IFN-γ and other related immunity-promoting substances that were evoked by GN. In addition, the contents of a specific protein that is produced in sera to immunosuppression of AC dogs are reduced with GN administration [6].
Furthermore, PHA skin-test anergic in the TB systems is closely correlated with NK activity. These unresolved events that require elucidation are now under study in our laboratories.

REFERENCES