Note

Administration of Anti-Asialo GM₁ Serum Increases Aberrant Crypt Foci Induced by 1,2-Dimethylhydrazine in the Large Bowel of Rats

Satoshi ISHIZUKA, Takumi NAGAI, and Takanori KASAI

Laboratory of Nutritional Biochemistry, Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan
(Received August 9, 1996)

Summary We investigated the influence of the administration of anti-asialo GM₁ antibody on aberrant crypt foci (ACF) formation induced by a single injection of 1,2-dimethylhydrazine (DMH). At four weeks after the injection of DMH (20 mg/kg body weight), the number of ACF and aberrant crypts were counted. Most ACF appeared in the distal large bowel, accounting for approximately 60% of the total ACF in both groups. Rats administered anti-asialo GM₁ had significantly more ACF in the distal colon, the rectum and the total large bowel as compared to control rats. A similar tendency was observed for the number of aberrant crypts. The increased number of ACF resulting from the administration of anti-asialo GM₁ was not accompanied by the enlargement of ACF size in every part of the colon. This study demonstrated that the administration of anti-asialo GM₁ at the initiation stage leads to an increase in ACF as well as aberrant crypts in the distal colon, rectum and total large bowel probably via the suppression of natural killer cells.

Key Words aberrant crypt foci, dimethylhydrazine, anti-asialo GM₁

The immune system, especially cytotoxicity mediated by natural killer (NK) cells and lymphokine-activated killer (LAK) cells, is believed to play an important role in anti-cancer defense mechanisms (1). A relationship between the stage of cancer and NK cytotoxicity has not been well defined. Furthermore, in patients with colorectal cancer, no reduction in NK activity was observed (2,3). In contrast, another report demonstrated that patients with low levels of NK cytotoxicity have a significantly increased risk of local recurrence (4). NK activity seems not to be effective against colorectal carcinogenesis at a later progressional stage because the tumor itself could have induced the production of suppressor cells or factors (5). Our question was whether or not natural cytotoxicity has a suppressive effect against initiated stem cells in the early stages of carcinogenesis of the colon. In animal models, the initial carcinogenic insult results in initiated stem
cells whose progeny remain under NK control (6, 7). In most colorectal tumorogenesis models, carcinogens such as 1,2-dimethylhydrazine (DMH) or azoxymethane were repeatedly injected into animals to form tumors (8). In procedures such as this, it is not only difficult to discriminate the initial stage from the promotional stage, but also much time and labor are required until tumorogenesis. There is also a risk of changing the cell proliferative features by repeated exposure to the carcinogen (8). We used aberrant crypt foci (ACF) as an index to assess natural cytotoxicity in the early stages of carcinogenesis. ACF proposed by Bird (9) are putative pre-neoplastic lesions and easily detectable by a simple methodological approach. They can be observed in the colon and rectum as early as two weeks after a single injection of DMH (10, 11).

In this report, we investigated the influence of the suppression of NK activity on ACF formation induced by DMH using anti-asialo GM1 as the suppressant.

This study was approved by the Hokkaido University Animal Use Committee, and animals were maintained according to the Guidelines for the Care and Use of Laboratory Animals, Hokkaido University. Twelve Wistar/ST rats were housed in individual cages in a temperature-controlled (23±2°C) room under a 12 h photoperiod (light 0800–2000). They were allowed free access to a standard diet (Table 1) and drinking water throughout the experimental period. After acclimatization, they were divided into two groups and intraperitoneally injected with either 100 µl of anti-asialo GM1 rabbit serum (Wako Pure Chemical Industries, Ltd., Osaka, Japan) or normal rabbit serum gammaglobulin (as the control) on days 1 and 4. On day 6, all rats were subcutaneously injected with N,N'-dimethylhydrazine (DMH, 20 mg/kg body weight). The DMH solution was prepared by dissolving N,N'-dimethylhydrazine dihydrochloride (Nacalai Tesque, Inc., Kyoto, Japan) in

Table 1. Composition of standard diet.

| Dietary component       | g/kg diet |
|-------------------------|-----------|
| Casein¹                 | 250       |
| Sucrose                 | 645       |
| Corn oil²               | 50        |
| Mineral mixture³        | 40        |
| Vitamin mixture⁴        | 10        |
| Vitamin E⁵              | 1         |
| Choline bitartrate      | 4         |

¹Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand). ²Retinyl palmitate (7.66 µmol/kg diet) and ergocalciferol (0.0504 µmol/kg diet) were added to corn oil. ³The mineral mixture is identical with MM2 described by Ebihara et al. (16). ⁴The vitamin mixture was prepared in accordance with the AIN-76 formulation (17), except that vitamin K as menadione and L-ascorbic acid were added to give 5.81 µmol/kg (18) and 284 µmol/kg (19) of the diet, respectively. ⁵Vitamin E (granulated, Juvela, Eisai Co., Tokyo, Japan) supplied 423 µmol all-rac-α-tocopheryl acetate/kg of the diet.

J. Nutr. Sci. Vitaminol.
1.5 mM EDTA saline and adjusting it to pH 6.5 with NaOH. At four weeks after DMH injection, all rats were sacrificed by exsanguination under anesthesia with diethyl ether. The large bowel (colon and rectum) was removed, flushed with cold saline and divided into four portions of nearly equal lengths. The four portions from proximal to anal verge were termed a proximal colon (PC), middle colon (MC), distal colon (DC) and rectum (RT). All the portions were slit open along the longitudinal axis and fixed flat on a board in 2% paraformaldehyde in phosphate-buffered saline (pH 7.5, PBS) at 4°C for 2 h. After washing with PBS, they were stained with 0.2% methylene blue for 30 min. Each portion was then placed on a slide glass with the mucosal surface up, and the number of ACF and aberrant crypts (AC) was counted.

Significant differences in the measured values between the two experimental groups were evaluated according to Hsu's Multiple Comparisons with Best. All statistical analyses were carried out using JMP computer software (SAS Institute Inc., NC, USA).

The administration of anti-asialo GM₁ did not influence growth parameters such as final body weight, weight gain, food intake and spleen weight (Table 2).

To elucidate whether reduced NK activity affects the formation of ACF, the influence of anti-asialo GM₁ on the formation of ACF induced by DMH was examined (Table 3). ACF appeared mostly in the distal colorectum (DC and RT), both in the group given anti-asialo GM₁ and the control group. The number of

| Table 2. Growth parameters in rats with or without anti-asialo GM₁ administration. |
|-----------------------------------|----------------|----------------|----------------|----------------|
|                                   | Initial body weight (g) | Final body weight (g) | Weight gain (g/33 days) | Food intake (g/day) | Spleen weight (g) |
| Anti-asialo GM₁                   | 243.1±5.1              | 443.5±14.4          | 200.4±11.0              | 23.3±0.5          | 0.877±0.044       |
| Control                           | 243.3±5.5              | 425.2±16.6          | 181.9±12.0              | 22.7±0.9          | 0.822±0.040       |

| Table 3. The number of aberrant crypt foci (ACF), aberrant crypts (AC) and AC/ACF in every part of the colorectum at four weeks after DMH injection. |
|-----------------------------------|----------------|----------------|----------------|----------------|
|                                   | PC       | MC       | DC       | RT       | Total     |
| Aberrant crypt foci              |          |          |          |          |           |
| Anti-asialo GM₁                  | n.d.     | 1.0±0.5  | 15.7±2.9*| 7.2±1.5*| 23.8±4.7*|
| Control                          | n.d.     | 1.5±1.0  | 8.2±1.7  | 3.3±0.6  | 13.0±3.0  |
| Aberrant crypt number            |          |          |          |          |           |
| Anti-asialo GM₁                  | n.d.     | 1.2±0.7  | 25.7±4.0*| 11.5±2.6*| 38.3±7.0  |
| Control                          | n.d.     | 2.5±1.4  | 13.8±4.3 | 5.0±1.2  | 21.3±6.3  |
| AC/ACF                           |          |          |          |          |           |
| Anti-asialo GM₁                  | —        | 0.6±0.3  | 1.7±0.1  | 1.6±0.1  | 1.6±0.1   |
| Control                          | —        | 1.0±0.5  | 1.6±0.2  | 1.4±0.1  | 1.5±0.1   |

*Significantly different from control, p<0.05, n=6.

n.d., not detected.
ACF in the DC accounted for approximately 60% of the total ACF in the whole large bowels of both groups. Rats given anti-asialo GM\(_1\) had significantly higher ACF in the DC, RT and whole large bowel than the control rats. Similar results were observed in the number of aberrant crypts in the DC and RT, respectively, but not in the total large bowel. This study demonstrated that the administration of anti-asialo GM\(_1\) at the initial stage causes an increase in both ACF and AC in the DC, RT and total large bowel.

It has been reported previously that NK activity is strikingly eliminated when anti-asialo GM\(_1\) is injected, and that the eliminated activity can be maintained by repeated injections at 3-day intervals (12), although it is still unclear whether such an increase in ACF is directly due to the inhibition of NK cells. The dose of anti-asialo GM\(_1\) used in this study was sufficient to induce the total elimination of the high-density fraction of large granular lymphocytes in the liver of Wistar rats (13), and to inhibit cytotoxicity of the peripheral blood lymphocytes for two weeks after injection (14). Although this dose of anti-asialo GM\(_1\) may not completely inhibit NK activity in the large bowel, the number of ACF in the distal large bowel increased significantly (Table 3). In animal models, reduced NK activity through the administration of anti-asialo GM\(_1\) or cyclophosphamide leads to the metastases of spontaneous and experimental tumors (1). These results suggest that the reduced NK activity allows the survival of stem cells damaged by carcinogens.

In both experimental groups, ACF with five or more crypts were not found (data not shown). The increase in ACF induced by the administration of anti-asialo GM\(_1\) was not accompanied by the enlargement of ACF size in every part of the colon. From the viewpoint of multiplicity, it may therefore be said that the administration of anti-asialo GM\(_1\) no longer affects the pre-neoplastic state. Regardless of the administration of anti-asialo GM\(_1\), the increase in ACF corresponded to the increase in AC.

In this study, we proposed the possibility that the formation of ACF was influenced by the immune system. In this connection, Carter et al. (15) discussed that lymphoid nodules in the distal large bowel play a promotional role following the initiation of colon carcinogenesis by repeated injections of a carcinogen. However, Bird (8) indicated that the dynamics of the disease process and the number and growth of ACF are significantly affected by the frequency of exposure to a carcinogen and the time when assessments are performed. The regulation of ACF or its subsequent tumor formation by the immune system may also be affected by repeated carcinogen injections.

In conclusion, the administration of anti-asialo GM\(_1\) increased the number of ACF and AC induced in the large bowel four weeks after the injection of DMH. This result supports the notion that the immune system, especially NK cells, plays an important role in the initial stage of colonic carcinogenesis.

We gratefully acknowledge Dr. Fumio Nakamura (Department of Animal Science, Faculty of Agriculture, Hokkaido University) for his helpful suggestions.

*J. Nutr. Sci. Vitaminol.*
REFERENCES

1) Brittenden, J., Heys, S. D., Ross, J., and Eremin, O. (1996): Natural killer cells and cancer. *Cancer*, 77, 1226–1243.

2) Aparicio, P. N., Verspaget, H. W., Pena, S. A., and Lamers, C. B. (1989): Impaired local natural killer cell activity in human colorectal carcinomas. *Cancer Immunol. Immunother.*, 28, 301–304.

3) Pislarasu, M., Oproiu, A., Taranu, D., Herberman, R. B., and Sulica, A. (1988): Modulation of natural killer cell activity by serum from cancer patients: preliminary results of a study of patients with colorectal adenocarcinoma or other types of cancer. *Cancer Res.*, 48, 2596–2603.

4) Tartter, P. I., Steinberg, B., Barron, D. M., and Martinelli, G. (1987): The prognostic significance of natural killer cytotoxicity in patients with colorectal cancer. *Arch. Surg.*, 122, 1264–1268.

5) Kumazawa, H., and Hess, M. (1991): Influence of serum derived from patients with head and neck cancer on natural killer cell activity. *Oncology*, 48, 372–376.

6) Altmann, G. G., Parhar, R. S., and Lala, P. K. (1990): Hyperplasia of mouse duodenal crypts and its control by NK cells during the initial phase of DMH carcinogenesis. *Int. J. Cancer*, 46, 695–702.

7) Altmann, G. G., and Lala, P. K. (1994): Initiated stem cells in murine intestinal carcinogenesis: prolonged survival, control by NK cells, and progression. *Int. J. Cancer*, 59, 569–579.

8) Bird, R. P. (1995): Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett.*, 93, 55–71.

9) Bird, R. P. (1987): Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett.*, 37, 147–151.

10) McLellan, E. A., and Bird, R. P. (1988): Aberrant crypts: Potential preneoplastic lesions in the murine colon. *Cancer Res.*, 48, 6187–6192.

11) Ishizuka, S., and Kasai, T. (1996): Inhibitory effect of dietary wheat bran on formation of aberrant crypt foci in rat colon induced by a single injection of 1,2-dimethylhydrazine. *Biosci. Biotech. Biochem.*, 60, 2084–2085.

12) Habu, S., Fukui, H., Shimamura, K., Kasai, M., Nagai, Y., Okumura, K., and Tamaoki, N. (1980): *In vivo* effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. *J. Immunol.*, 127, 34–38.

13) Vanderkerken, K., Bouwens, L., De Neve, W., van den Berg, K., Baekeland, M., Delens, N., and Wisse, E. (1993): Origin and differentiation of hepatic natural killer cells (pit cells). *Hepatology*, 18, 919–925.

14) Shimizu, T., Pelletier, H., Hammann, A., Olsson, N. O., Martin, M. S., and Martin, F. (1987): Effects of a single injection of anti-asialo-GM1 serum on natural cytotoxicity and the growth of a regressive colonic tumor in syngeneic rats. *Int. J. Cancer*, 40, 676–680.

15) Carter, J. W., Lancaster, H. K., Hardman, W. E., and Cameron, I. L. (1994): Distribution of intestine-associated lymphoid tissue, aberrant crypt foci, and tumors in the large bowel of 1,2-dimethylhydrazine-treated mice. *Cancer Res.*, 54, 4304–4307.
16) Ebihara, K., Imamura, Y., and Kiriyama, S. (1979): Effect of dietary mineral composition on nutritional equivalency of amino acid mixture and casein in rats. *J. Nutr.*, **109**, 2106–2116.

17) American Institute of Nutrition (1977): Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.*, **107**, 1340–1348.

18) American Institute of Nutrition (1980): Second report of the ad hoc committee on standards for nutritional studies. *J. Nutr.*, **110**, 1726.

19) Harper, A. E. (1959): Amino acid balance and imbalance. 1. Dietary level of protein and amino acid imbalance. *J. Nutr.*, **68**, 405–418.