Vitamin A and tumor immunity[1,2]

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Summary. Intraperitoneal administration of vitamin A into the BALB/c mice inoculated with a syngeneic fibrosarcoma, Meth A, caused a remarkable augmentation of tumor rejection. A cell-depletion technique revealed that the primary effector cells responsible for the augmented rejection were Thy-1 positive, Lyt-1 negative, Lyt-2 positive lymphocytes, suggesting the involvement of cytotoxic lymphocytes.
Key words. Mouse, BALB/c; fibrosarcoma Meth A; vitamin A, tumor rejection; lymphocytes, cytotoxic.

Many experimental data have demonstrated the antitumor effect of vitamin A[3]. This effect may be in part ascribed to the adjuvanticity of vitamin A[4–6]. Vitamin A was reported to enhance the immunological rejection against tumor cells[7–9], although there was no concrete evidence about the precise mechanism for the augmentation of tumor immunity by vitamin A or details about the precise nature of the effector cells. We have also examined the effect of vitamin A on tumor rejection, using a syngeneic tumor system of Meth A fibrosarcoma and BALB/c mice and have demonstrated that the administration of vitamin A augmented the tumor rejection which is specific to tumor-specific antigen[13]. This paper describes the nature of the effector cells causing an enhanced tumor rejection by vitamin A in the same system.

Materials and methods. Female BALB/c mice (Shizuoka Laboratory Animal Center, Shizuoka) were kept on the basal diet CE-II (CLEA Japan Inc., Tokyo), and were used for the experiments at 8 weeks of age. Meth A fibrosarcoma (Meth A) was maintained by weekly passage in the peritoneal cavities of BALB/c mice. Chocola A (retinol palmitate) purchased from Eisai Co. (Tokyo) was used as vitamin A (RP). RP was dissolved in a phosphate-buffered saline (0.8% NaCl, 0.02% KCl, 0.11% Na_2HPO_4, 0.02% KH_2PO_4) before injection (0.2 ml/mouse). In our preceding paper[13], the dose of 5000 IU/kg/day was shown to be suitable for the experiments on tumor immunity. The suppressive effect of immune lymph node cells (LNC) on tumor growth was assayed by the Winn assay, as follows. BALB/c mice were inoculated s.c. with 10^7 Meth A cells on day 0 and were given i.p. RP from day 1 to day 9. The control group was given only solvent after the tumor inoculation. LNC were obtained from these mice on day 12 and mixed with Meth A cells. The mixture containing 4×10^6 LNC and 10^6 Meth A cells was inoculated s.c. into healthy BALB/c mice. The growth of the Meth A tumor was measured on the days indicated in tables 1 and 2. To identify the phenotype of the effector cells, antisera-treatment of the LNC was carried out before mixing LNC with Meth A cells for the Winn assay. The LNC obtained from the Meth A-inoculated, RP-treated mice were treated with antisera (anti-Thy-1.1 alloantiserum, anti-Lyt-1.2 alloantiserum, anti-Lyt-2.2 monoclonal antibody: Cedarlane Laboratories Ltd.) for 45 min at 4°C and further incubated with complement (Low-Tox-M-rabbit complement: Cedarlane Laboratories Ltd.) for 45 min at 37°C.

Results and discussion. The growth of Meth A was suppressed slightly by the LNC from the Meth A-inoculated BALB/c mice without RP-treatment (table 1). By administering RP into the tumor-inoculated mice, the LNC caused a remarkable retardation of the tumor growth. Suppressive effect of the LNC obtained from the Meth A-inoculated, RP-treated mice on the growth of tumor was abolished by the treatment with anti-Thy-1.2 or anti-Lyt-2.2, but was not affected by the treatment with anti-Lyt-1.2 in the presence of complement (table 2). The immunological tumor rejection augmented by RP-treatment appears to be due primarily to the lymphocytes bearing Thy-1^+, Lyt-1^-,2^+ cell surface antigens, presumably cytotoxic T lymphocytes. In contrast to our results, cytostasis has been supposed to play an important role in the rejection of syngeneic tumor cells[10–12]. Furthermore, allograft-rejection augmented with vitamin A acetate was due to the induction of Lyt-1^+ lymphocytes associated with delayed-type hypersensitivity[14]. On the other hand, Lotan et al.[7–9] reported that cytotoxic T lymphocytes were induced by retinoic acid in allogeneic and syngeneic tumor systems, although the phenotype of the effector cells was not clearly identified. Our finding is in agreement with their observation. However, the present evidence is not conclusive as to whether different type of effector cells may be induced by vitamin A in different tumor systems.

Table 1. Tumor growth of Meth A cells with Meth A-primed, RP-treated LNC[a]

Group	Tumor growth [mm²][b] Day 6	Day 11
Normal (untreated mice)	66.06 ± 7.19	135.77 ± 7.88
Control (Meth A-primed mice)	56.77 ± 3.84	107.07 ± 12.50
RP (Meth A-primed, RP-treated mice)	36.54 ± 3.76*	59.92 ± 6.43*

[a] BALB/c mice were inoculated s.c. with 10^7 Meth A cells on day 0 and given i.p. RP from day 1 to day 9. LNC were obtained from these mice on day 12 and mixed with Meth A cells. The mixture containing 4×10^6 LNC and 10^6 Meth A cells was inoculated s.c. into normal BALB/c mice. The growth of Meth A tumor was measured on the days indicated in the table. Each group consisted of 8 mice. [b] Tumor growth was recorded as the product of the longest and shortest diameters (mean ± SE). * Statistical significance: $p < 0.005$ vs normal.

Table 2. Effect of anti-Lyt-treatment on the neutralization test with Meth A-primed, RP-treated LNC[a]

Anti-Lyt-treatment	Tumor growth [mm²][b]	
Experiment 1	Day 8	Day 14
No treatment	33.44 ± 9.16	49.45 ± 26.06
Anti-Thy-1.2 + complement	61.63 ± 4.70*[2]	130.71 ± 23.65*
Complement	33.33 ± 5.58	61.31 ± 17.63
Experiment 2	Day5	Day 10
No treatment	31.10 ± 5.61	79.84 ± 8.20
Anti-Lyt-1.2 + complement	18.97 ± 3.62	62.70 ± 8.94
Anti-Lyt-2.2 + complement	49.27 ± 3.54**	124.11 ± 4.84***
Complement	30.23 ± 3.30	76.65 ± 14.54

[a] BALB/c mice were inoculated with Meth A and treated with RP as described in table 1. LNC were obtained from these mice on day 12 and treated with antisera plus complement before the neutralization test. The growth of Meth A tumor was measured on the days indicated in the table. Each group consisted of 8 mice. [b] Tumor growth was recorded as the product of the longest and shortest diameters (mean ± SE). * Statistical significance: $p < 0.05$ vs no treatment. ** Statistical significance: $p < 0.02$ vs no treatment. *** Statistical significance: $p < 0.005$ vs no treatment.

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Differential binding of conA and WGA on the cell surface, the role of sialic acid in their expression and the increased activity of sialidase after cis-Platin treatment

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Summary. It is reported that concanavalin A (conA) and wheat germ agglutinin (WGA) have a differential binding pattern on normal mouse spleen lymphocytes and the surface of Dalton's lymphoma cells. It is suggested that sialic acid on the cell surface controls the expression of lectin binding sites. Further, it has been observed that the increased release of sialic acid from cell surfaces after *cis*-dichlorodiammine platinum (II) (cis-Platin) treatment is due to the increased activity of sialidase.
Key words. Mouse spleen cell; mouse lymphoma; concanavalin A binding; wheat germ agglutinin binding; sialic acid.

cis-Dichlorodiammine platinum (II) (cis-Platin) a potent antitumor compound[2-5] has a definite effect on the surface of tumor and normal cells, and particularly affects lectin binding sites and cell agglutination. Normal lymphocytes (NL), which agglutinate very little with conA and WGA show weak fluorescence with lectin-FITC (fluorescein isothiocyanate). NL when incubated with cis-Platin show a gradual increase in the degree of cell agglutination with concanavalin A (conA) and wheat germ agglutinin (WGA), and a significant increase in the fluorescence intensity with lectin-FITC is observed after 10–30 min of treatment. Maximum cell agglutination and fluorescence intensity are observed after 30 min of treatment, and 60 min treatment results in sharp decrease in both cell agglutination and fluorescence intensity, to the level of untreated NL. On the other hand, Dalton's lymphoma (DL) cells, which show a high degree of cell agglutination with lectins and bright fluorescence with lectin-FITC, when treated with cis-Platin gradually show a significant decrease in the cell agglutination and fluorescence intensity from 10 to 60 min of treatment. The least cell agglutination and weakest fluorescence are observed after 60 min of treatment[6-8]. Various ultrastructural and biochemical studies have shown that in vivo and in vitro cis-Platin treatment of the cells causes a gradual removal of cell surface sialic acid and mucopolysaccharides[6,7,9]. This removal of sialic acid from the cell surface was observed to have a role in the differential increase or decrease in the degree of agglutination of the cells with lectins, and neuraminidase treatment of normal and tumor cells resembled closely the results seen with cis-Platin[6,7]. The present fluorescence and biochemical studies were undertaken to provide direct evidence for a) the different binding patterns of conA and WGA to the cell surfaces, b) the role of sialic acid in the expression of lectin binding sites on normal and tumor cells, and c) the possible mechanism of release of sialic acid from the cell surfaces after cis-Platin treatment.

It is reported that there is a differential binding behavior of conA and WGA on cell surfaces; the presence of sialic acid moieties is apparently correlated with the expression of lectin binding sites and removal of sialic acid from the cell surface seems to be due to an increase in sialidase activity after cis-Platin treatment.

Materials and methods. Single-cell suspensions of normal spleen lymphocytes and Daltons's lymphoma ascites cells from Dba mice were prepared in PBS (without Ca^{++} and Mg^{++}) as described earlier[7]. Ganglioside (type IV), conA-FITC, WGA-FITC, neuraminidase (type V), sialic acid (type VI) were purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade; cis-Platin was thoroughly mixed in 0.89% NaCl in the dark 10–15 min before the experiments. For fluorescence microscopy the cell count was adjusted to 2×10^6 cells/ml. 3.0 ml of cell suspensions were incubated with cis-Platin (25 µg/ml) or with neuraminidase (2.5 units/ml) and without cis-Platin or neuraminidase (controls) for 20 and 30 min at 37 °C. After incubation, the cell suspensions were centrifuged and the pellets were suspended in one fourth of the original volume of PBS, fixed in 2% glutaraldehyde and labelled with conA-FITC (100 µg/ml) or WGA-FITC (50 µg/ml) as previously described[7]. In another set of experiments NL and DL cells were treated with cis-Platin (25 µg/ml) or neuraminidase (2.5 units/ml) for 30 and 60 min, respectively. After incubation, the cells were centrifuged and the pellets were suspended in the same volume of PBS containing 2 mg/ml of sialic acid for 20 min at room temperature. The cells were washed twice with PBS and then processed for fluorescence microscopy.

For sialidase assay in NL and DL cells (4×10^6 cells/ml) treated with cis-Platin (25 µg/ml) for 30 min at 37 °C or without cis-Platin (controls) the method of Schengrund and Rosenberg[10] was used with some modifications: duplicate samples of the cell suspensions (1.0 ml) were centrifuged and the pellets were suspended in 1.0 ml of 0.02 M acetate buffer, pH 4.0, containing different concentrations (100, 200, and 500 µg/ml) of substrate (brain ganglioside type IV). A substrate blank and an enzyme blank were also prepared omitting the substrate or the cells from the reaction mixture. The reacting mixture was incubated for 90 min at 37 °C. The reaction was stopped by adding 0.15 ml of 0.1 N NaOH. Then the cell suspensions were