Brief Communication: Promotion of Incidence of Adenovirus Type 12 Transplantable Tumors by Carrageenan, a Specific Antimacrophage Agent[1,2]

Eva Lotzová, *Ph.D.*,[3,4] *and* **Ellen R. Richie**, *Ph.D.*[3,5,6]

ABSTRACT—Carrageenan, a sulfated polygalactose with known macrophage-toxic properties, was used to ascertain the role of macrophages in resistance to adenovirus type 12 transplantable tumors. A single ip injection of 5 or 10 mg carrageenan led to increased incidence and more rapid growth of tumors in C3H mice. Carrageenan was most effective if given 1 day before tumor inoculation; the effectiveness decreased with increasing intervals before or after tumor cell injection. The macrophage stabilizer poly-2-vinylpyridine *N*-oxide injected sc (150 mg/kg) 1 day before carrageenan was given reduced the incidence of tumors. These data lend further support to the importance of macrophages in tumor immunity.—J Natl Cancer Inst 58: 1171–1172, 1977.

The function of macrophages in relation to tumor growth has recently caused much excitement. A number of reports have indicated a specific (*1, 2*) as well as a nonspecific (*3*) role of macrophages in the prevention of tumor growth. Evidence that macrophages may play a singificant role in tumor immunity is based on the observations that macrophages accumulate at the sites of inoculation of tumor cells (*4*); that immune macrophages are cytotoxic in vitro (*1, 2*); that the impairment of macrophage function by antimacrophage serum leads to enhancement of tumor growth (*5*); and that trypan blue and gold salts, the agents inhibiting lysosomal enzyme activity of macrophages, abrogate or decrease specific and nonspecific response to tumor growth in experimental animals (*6, 7*).

In the present study the role of macrophages in resistance to Ad-12 transplantable tumors in mice was investigated by the use of carrageenan, an agent reported to be toxic specifically for macrophages and not for lymphocytes (*8–10*). The results presented here strongly suggest that macrophages are one of the cell types necessary for rejection of Ad-12 transplantable tumors.

MATERIALS AND METHODS

Mice.—Specific pathogen-free, 2- to 4-month-old inbred C3H females were provided by Dr. John Trentin.

Tumor.—Tumors were induced by the inoculation of newborn C3H mice sc with 0.05 ml Ad-12. The single tumor line was then maintained by serial transplantation of 3×10^6 tumor cells in suspension. To detect the effect of carrageenan on development of tumors, only 10^6 tumor cells were given to each recipient mouse to lower tumor incidence.

Macrophage-modulating agents.—Carrageenan (Sea-Kem 9) was obtained from Dr. D. Renn, Marine Colloids Inc., Rockland, Maine. It was dissolved in saline by being heated in a hot water bath and injected ip in doses of 5 or 10 mg/mouse. PVNO was obtained from Polysciences Inc., Warrington, Pennsylvania, and was suspended in saline and injected sc at a dose of 150 mg/kg. PVNO was injected 1 day prior to injection of carrageenan.

RESULTS

In the first series of experiments, C3H mice were given 5 or 10 mg carrageenan ip. There was no difference in the effect of these two doses; therefore, the data were pooled. Carrageenan was administered either 1–3 days before or 1–3 days after inoculation of 10^6 tumor cells. Control groups were given tumor cells only. The animals were checked for tumor incidence at different intervals after tumor inoculation.

None of 20 untreated mice developed tumors at day 15, and only 4 of 20 (20%) and 5 of 20 (25%) developed tumors at day 24 and 38, respectively (table 1). In contrast, mice given carrageenan were more susceptible to tumor growth. If carrageenan was given 1 day prior to tumor inoculation, 12 of 24 mice (50%) developed tumors at day 15, 17 of 22 (77%) at day 24, and 19 of 22 (86%) at day 38. The difference between untreated mice and those treated with carrageenan 1 day before tumor cell injection was highly statistically significant. Mice given carrageenan 3 days before or 1–3 days after tumor cell inoculation also had a higher incidence of tumors than did untreated controls.

In the second series of experiments, we attempted to reduce the effect of carrageenan by PVNO, the macrophage-stabilizing agent (*11*). PVNO presumably stabilizes the macrophage lysosomes and thus prevents their disruption and cellular death. It was, therefore, expected that PVNO administered before carrageenan would protect macrophages from the toxic effect of carrageenan and thus decrease the incidence of tumors (table 2).

PVNO reduced the number of tumor-positive animals. There was no statistically significant difference between mice treated with PVNO plus carrageenan and untreated groups, whereas the difference between untreated and carrageenan-treated groups was highly significant statistically. The difference in the percentage of neoplasms in untreated controls between tables 1 and 2 was a reflection of variability in tumor-producing poten-

ABBREVIATIONS USED: Ad-12 = adenovirus-12; PVNO = poly-2-vinylpyridine *N*-oxide.

[1] Received July 29, 1976; accepted October 14, 1976.

[2] Supported by Public Health Service grants CA12093, CA03367, and K6CA14219 from the National Cancer Institute.

[3] Division of Experimental Biology, Baylor College of Medicine, Houston, Tex. 77030.

[4] *Address reprint requests to* Dr. Lotzová at her *present address*: Department of Developmental Therapeutics, M. D. Anderson Hospital, The University of Texas System Cancer Center, Texas Medical Center, Houston, Tex. 77030.

[5] Department of Pediatrics, The University of Texas System Cancer Center.

[6] We thank Dr. Karel Dicke for reading the manuscript and the helpful suggestions and discussions and Dr. John Trentin for providing us with mice, adenovirus-12, and facilities.

TABLE 1.—*Effect of carrageenan on the growth of Ad-12 transplantable tumors*

Time after inoculation of 10^6 tumor cells[a] days	Day of carrageenan treatment[b]	Number positive/total No. of tumors	Percent of tumor-bearing mice	Fisher exact probability[c]
15	None	0/20	0	
	−3	1/10	10	NS
	−1	12/24	50	0.0003
	+1	2/22	9	NS
	+3	3/13	23	0.05
24	None	4/20	20	
	−3	4/10	40	NS
	−1	17/22	77	0.0003
	+1	9/18	50	0.05
	+3	6/13	46	NS
38	None	5/20	25	
	−3	6/10	60	NS
	−1	19/22	86	0.0001
	+1	13/22	59	0.03
	+3	6/13	46	NS

[a] Tumor inoculation on day 0.
[b] Carrageenan given ip in doses of 5 and 10 mg/mouse; results for both doses were pooled.
[c] NS=not significant.

TABLE 2.—*Effect of carrageenan and PVNO on the growth of Ad-12 transplantable tumors*

Time after inoculation of 10^6 tumor cells[a] days	PVNO treatment[b]	Carrageenan treatment[c]	Number positive/total No. of tumors	Percent of tumor-bearing mice	Fisher exact probability[d]
24	No	No	0/10	0	
	No	Yes	4/9	44	0.03
	Yes	Yes	2/10	20	NS
35	No	No	0/10	0	
	No	Yes	6/9	67	0.003
	Yes	Yes	3/10	30	NS
63	No	No	1/10	10	
	No	Yes	6/9	67	0.01
	Yes	Yes	4/10	40	NS

[a] Tumor inoculation on day 0.
[b] PVNO given sc at a dose of 150 mg/kg.
[c] Carrageenan given ip in the dose of 5 mg/mouse.
[d] NS=not significant.

tial of the same number of cells obtained from different tumor pools.

Preliminary results with silica (particles of average size, <5 μ), another selective antimacrophage agent (*8, 12*), confirmed the results obtained with carrageenan, namely, that the incidence of tumors in animals pretreated with silica 1 day before inoculation of tumor cells was significantly higher than that in untreated mice. And again, PVNO given 24 hours before silica reduced the number of tumor-positive mice.

DISCUSSION

Carrageenan was reported to suppress delayed hypersensitivity skin reaction (*13, 14*) and to prolong rejection of skin allografts (*11*). Both these effects were attributed to a toxic effect specifically against macrophages as opposed to lymphoid cells (*8, 12*). In addition, carrageenan was found to interfere with macrophage processing of antigen (*15*). In the present study, a single ip injection of carrageenan reduced the ability of mice to resist 10^6 Ad-12-induced transplantable tumors. Carrageenan was most effective if given 24 hours before tumor cell injection.

The macrophage stabilizer PVNO is known to counteract the effect of carrageenan; e.g., it prevents the prolongation of skin allografts by carrageenan (*11*). Similarly, in our experiments, PVNO given 1 day prior to carrageenan reduced the incidence of tumor growth. Both these findings—the higher incidence of tumor-positive mice after treatment with carrageenan and the ability of PVNO to reduce this effect—are convincing evidence that macrophages play an important role in the rejection of Ad-12 transplantable tumors.

REFERENCES

(1) EVANS R, ALEXANDER P: Cooperation of immune lymphoid cells with macrophages in tumour immunity. Nature 228:620–622, 1970

(2) ———: Mechanism of immunologically specific killing of tumour cells by macrophages. Nature 236:168–170, 1972

(3) KELLER R, JONES VE: Role of activated macrophages and antibody in inhibition and enhancement of tumour growth in rats. Lancet 2:847–849, 1971

(4) GERSHON, RK, CARTER RL, LANE NJ: Studies on homotransplantable lymphomas in hamsters. IV. Observations on macrophages in the expression of tumor immunity. Am J Pathol 31:1111–1133, 1967

(5) ISA AM, SANDERS BR: Enhancement of tumor growth in allogeneic mice following impairment of macrophage function. Transplantation 20:296–302, 1975

(6) HIBBS JB: Activated macrophages as cytotoxic effector cells. I. Inhibition of specific and nonspecific tumor resistance by trypan blue. Transplantation 19:77–81, 1975

(7) MCBRIDE WH, TUACH S, MARMION BP: The effect of gold salts on tumour immunity and its stimualtion by *Corynebacterium parvum*. Br J Cancer 32:558–567, 1975

(8) ALLISON AC, HARINGTON JS, BIRBECK M: An examination of the cytotoxic effects of silica on macrophages. J Exp Med 124:141–153, 1966

(9) CANTAZARO PJ, SCHWARTZ HJ, GRAHAM RC: Spectrum and possible mechanism of carrageenan cytotoxicity. Am J Pathol 64:387–399, 1971

(10) LAKE WW, BICE D, SCHWARTZ HJ, et al: Suppression of in vitro antigen-induced lymphocyte transformation by carrageenan, a macrophage-toxic agent. J Immunol 107:1745–1751, 1971

(11) RIOS A, SIMMONS RL: Poly-2-vinylpyridine *N*-oxide reverses the immunosuppressive effects of silica and carrageenan. Transplantation 13:343–345, 1972

(12) PEARSALL NN, WEISER RS: The macrophage in allograft immunity. I. Effects of silica as a specific macrophage toxin. J Reticuloendothel Soc 5:107–120, 1968

(13) SCHWARTZ HJ, LESKOWITZ S: The effect of carrageenan on delayed hypersensitivity reactions. J Immunol 103:87–91, 1969

(14) BICE D, SCHWARTZ HJ, LAKE WW, et al: The effect of carrageenan on the establishment of delayed hypersensitivity. Int Arch Allergy Appl Immunol 41:628–636, 1971

(15) BICE DE, GRUWELL DG, SALVAGGIO JE, et al: Suppression of primary immunization by carrageenan—a macrophage toxic agent. Immunol Commun 1:615–625, 1972