

**An acidic polysaccharide Chlon A, from Chlorella
Pyrenoidosa**

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An acidic polysaccharide, chlon A, from *Chlorella PYrenOidOsa*

2. Antitumor activity and immunological response

Chlon A, an acidic polysaccharide purified from the hot water extract of *Chlorella pyrenoidosa*, possessed antitumor activity against transplantable murine tumors in vivo. In allogeneic systems, Chlon A showed remarkable life prolongation effects in mice bearing Sarcoma 180 with a broad optimal dose range. Chlon A was also active in syngeneic systems such as IMC carcinoma, Meth A fibrosarcoma, B16 melanoma and Lewis lung carcinoma growing in the intraperitoneal cavity. When Meth A cells were admixed with Chlon A and inoculated sc, remarkable growth inhibition of tumor cells was observed. Chlon A enhanced cytotoxicity of mice macrophages for EL-4 tumor cells in vivo, lymphoproliferative effects in vitro, and carbon clearance activity (reticuloendothelial system) in vivo. The electrophoretic pattern showed that LA (ceruloplasmin) and/or LB (hemopexin)like components rapidly appeared in serum proteins of mice receiving Chlon A. These findings indicate that Chlon A is a potent modifier of some biological responses.

Table 2. Antitumor activity of Chlon A on Sarcoma 180 and Meth-A fibrosarcoma.

Mice were given with Chlon A from days 1 to 5 and 7 to 11.

a) Numbers in parentheses indicate the number of cured mice/seven treated mice. Cured mice were excluded from the calculation of ILS (%).

b) Zymosan was purchased from SIGMA Chemical Company (ST. Louis, USA).

Table 3. Antitumor activity of Chlon A on IMC carcinoma, P388 leukemia Lewis lung carcinoma and B-16 melanoma.

Mice were given with Chlon A from days 1 to 5 and 7 to 11.

Table 4. In vivo growth of Meth A cells mixed with Chlon A.

Meth A cells suspended in minimum essential medium were admixed with Chlon A in vitro, and incubated for 30 minutes at 37°C. After incubation, tumor cells were inoculated subcutaneously into BALB/c mice, and tumor growth was measured periodically by calipers.

Table 5. Lymphoproliferative effect of Chlon A.

Spleen cells from normal mice were mixed with Chlon A, incubated for 72 hours at 37°C, and the uptake of ³H-TdR into spleen cells was measured.

Table 6. Carbon clearance in mice treated with Chlon A.

Normal ddY mice received Chlon A (once a day for 2 days, iv). On day 3, mice were injected iv with India ink (Pelican Fount India) and blood was withdrawn periodically from the retro-orbital plexus. Phagocytic activity was measured by the method of Halpern³).

Fig. 1. Antitumor activity of Chlon A on Sarcoma 180 solid tumors.

Mice were given Chlon A iv on days 1, 3, 5, 7 and 9.

Fig. 2. Effect of Chlon A on the cytolytic activity of peritoneal exudate cells.

Adherent peritoneal exudate cells (PEC) of ddY mice were collected on day 1 or 4 after injection of Chlon A and were mixed with EL-4 cells previously labeled with ³H-uridine. After incubation for 16 hours, the radioactivity of EL-4 cells was measured. (1-T/C) x 100 (%). The mean radio-activity of the control group was 5,931 cpm.

Fig. 3. Effect of Chlon A on the cytostatic activity of peritoneal exudate cells.

Adherent peritoneal exudate cells (PEC) of ddY mice were collected on day 1 or 4 after injection of Chlon A. Then ³H-TdR (1 ug/ml) was added to the cell mixture, and incubation was further continued for

5 hours. The uptake of ³H-TdR into EL-4 cells was measured. (1 -T/C) x 1 00 (%). The mean radioactivity of the control group was 6,332 cpm.

Fig. 4. Effect of Chlon A on the electrophoretic pattern of mouse serum.

Chlon A was injected iv into normal ddY mice on day 0, and sera were obtained on days 3, 5, 7, and 9. Sera were subjected to polyacrylamide gel electrophoresis on gradient gel. After electrophoresis, the gel was stained with 15 Amidoblack 10B solution.

TABLE 1 Tumor used in this Experiment			
Tumor	Animal	Inoculum size	
		cells/mouse	Inoculum site
Sarcoma 180	ICR 6w female	1 X 10 ⁵	ip or sc
IMC carcinoma	CDF ₁ 6w female	1 X 10 ⁵	ip
Meth A fibrosarcoma	BALB/c 6w female	1 X 10 ⁶	ip or sc
B16 melanoma	C ₅₇ BL 6w female	1 X 10 ⁶	ip
Lewis lung carcinoma	C ₅₇ BL 6w female	1 X 10 ⁶	ip
P388 leukemia	CDF ₁ 6w female	1 X 10 ⁵	ip

TABLE 2 Antitumor activity of Chlon-A on Sarcoma 180 and Meth-A fibrosarcoma (ip-ip)									
Sample	Total dose (mg/kg)	Sarcoma 180				Meth -A fibrosarcoma			
		MSD	(range)	ILS	(%)	MSD	(range)	ILS	(%)
Saline	---	13.9	(11-18)	---	0	13.3	(13-14)	---	0
Zyosan ^{b)}	20 x 10	20.3	(13-34)	46	(1)	15.4**	(15-16)	15.8	(0)
	5 x 10	17.1*	(15-22)	23	(0)	15.6**	(14-17)	17.3	(0)
Chlon-A	100 x 10	22.9**	(10-43)	64.7	(0)	17.8**	(16-19)	33.8	(1)
	25 x 10	31**	(15-41)	123	(2)	20**	(17-25)	50.4	(0)
	6.25 x 10	49.5**	(39-54)	256	(1)	20**	(15-27)	50.4	(0)
	1.56 x 10	26.3**	(12-57)	89	(1)	15.6	(12-17)	17.3	(0)

*p < 0.05 **p < 0.01

Numbers in parenthesis indicate number of cured mice/7 treated mice.

Cured mice were excluded from the calculation of ILS (%).

Zyosan was purchased from SIGMA chemical company (ST. Louis, USA).

TABLE 3 Antitumor activity of Chlon-A on IMC carcinoma, P388 leukemia, Lewis lung carcinoma and B-16 melanoma (ip-ip)

		IMC carcinoma		P388 leukemia		Lewis lung carcinoma		B-16 melanoma	
Sample	Total dose (mg/kg)	MSD (range)	ILS (%)	MSD (range)	ILS (%)	MSD (range)	ILS (%)	MSD (range)	ILS (%)
Saline	---	14.8 (14-16)	---	12	---	18.2 (10-22)	---	16.2 (14-18)	---
Chlon-A	100 x 10	24.4** (20-23)	44.6	1.1	-8.3	25.8* (22-30)	41.8	24** (20-31)	48.1
	25 x 10	21.6** (20-25)	45.9	12.2 (11-13)	1.7	21.8 (15-27)	19.8	21.4** (18-24)	32.1
	6.25 x 10	22.4** (20-23)	51.4	1.1	-8.3	23.8 (21-27)	30.5	23** (19-26)	42
	1.56 x 10	20.6** (20-23)	39.2	11.2 (11-12)	-6.7	20.6 (17-23)	13.2	23.6* (19-31)	45.7

*p < 0.05 **p < 0.01

TABLE 4
In vivo growth of Meth-A cells mixed with Chlon A

Dose (mg/kg)	Days	Tumor size (mm ²)			
		7	14	21	27
Saline		68.4	234.4	510	686.6
200		±**	24.6** (90)	84.3* (83)	175.6** (74.4)
40		5.7** (91.7)	47.6** (79.7)	159** (69)	331** (51.8)
8		14.57** (78.7)	75.3** (67.9)	208.4* (59)	445.1** (35.2)
1.6		32.7** (62.2)	124.1** (47.1)	382.7 (25)	631.8 (8)

*p < 0.05 **p < 0.01

Numbers in parenthesis indicate percent inhibition of tumor growth.

TABLE 5
Lymphoproliferative effect of Chlon-A

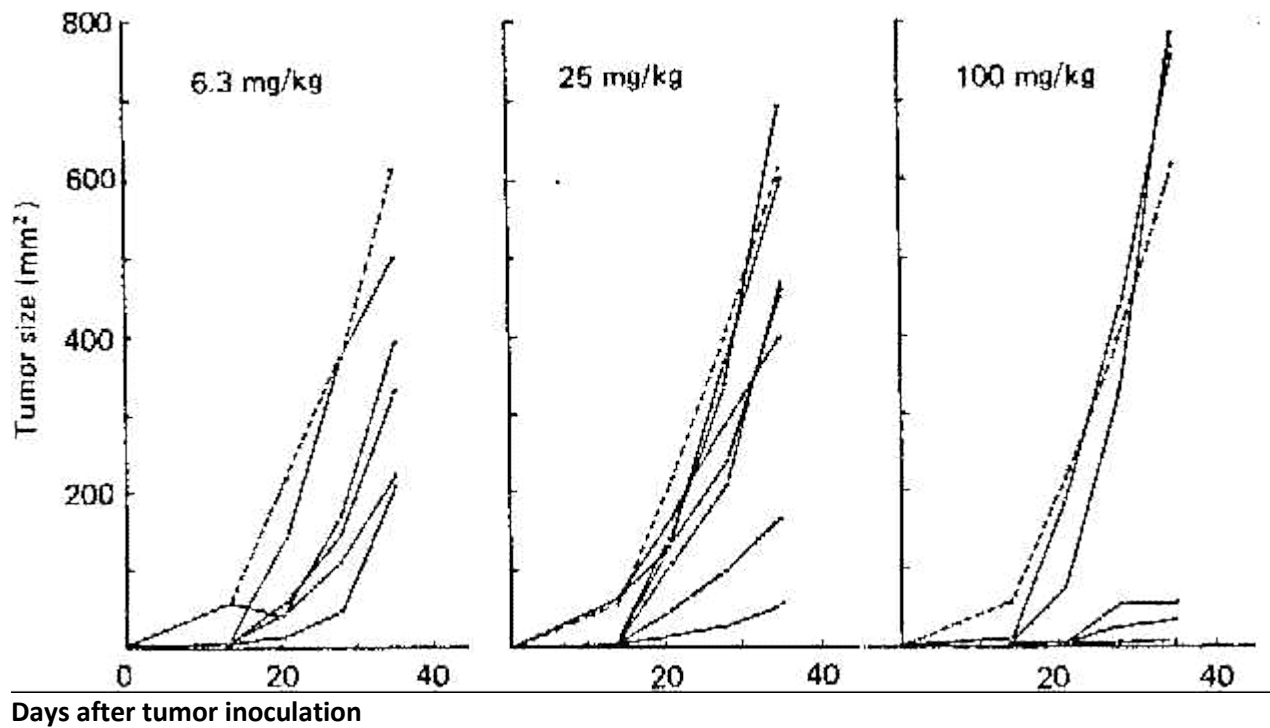
Sample	Dose (ug/ml)	H-TdR count (cpm)	Stimulation index
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Saline		902.3	1
Con A	5	14988.2	16.6
	1	83046.5	92
LPS	50	18097.2	20
	12.5	22856.9	25.3
Chlon-A	1000	22208.5	24.6
	250	17534.4	19.4
	62.5	7992.7	8.9
	15.6	5761.5	6.4
	3.9	3456.7	3.4

Total dose (mg/kg)	Phagocytic index (k)	spleen weight (mg)	Liver weight (g)
---	0.0079	113 ± 21	1.495 ± 0.09
100	0.0086 ± 0.002	197 ± 27	1.478 ± 0.07
25	0.0105 ± 0.001	205 ± 22	1.420 ± 0.08
6.25	0.0113* ± 0.001	185 ± 24	1.580 ± 0.1

*p < 0.1

Fig. 1 Effect of Chlon A on Sarcoma 180 Solid Tumor



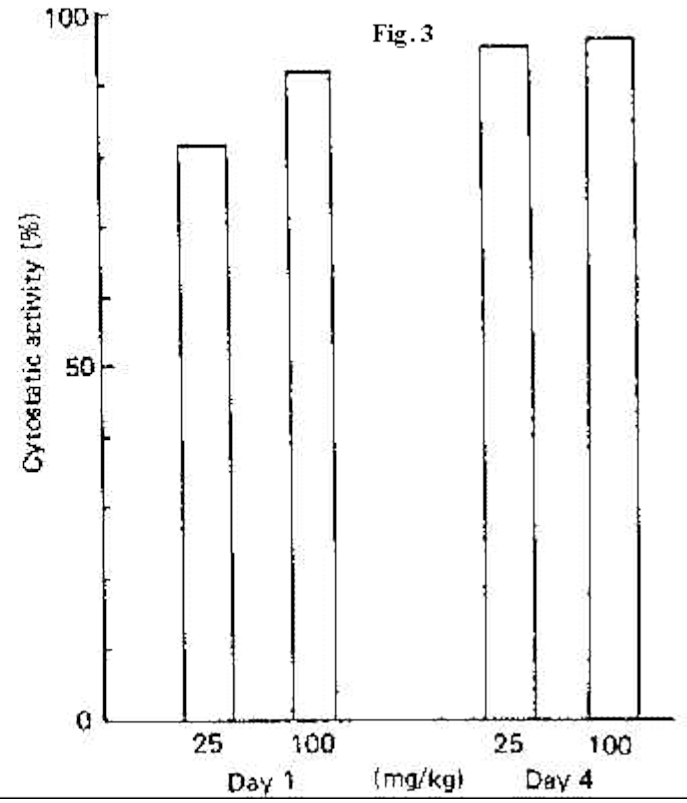
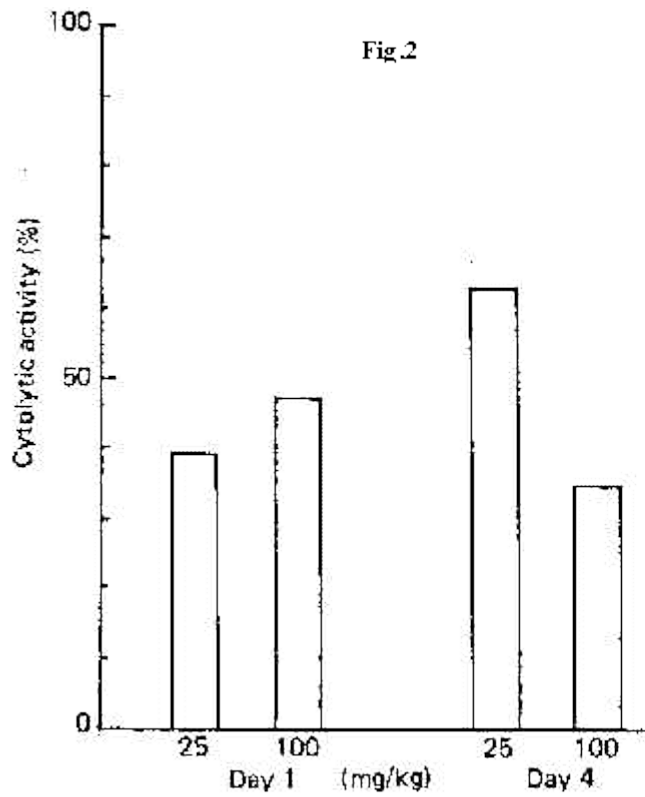
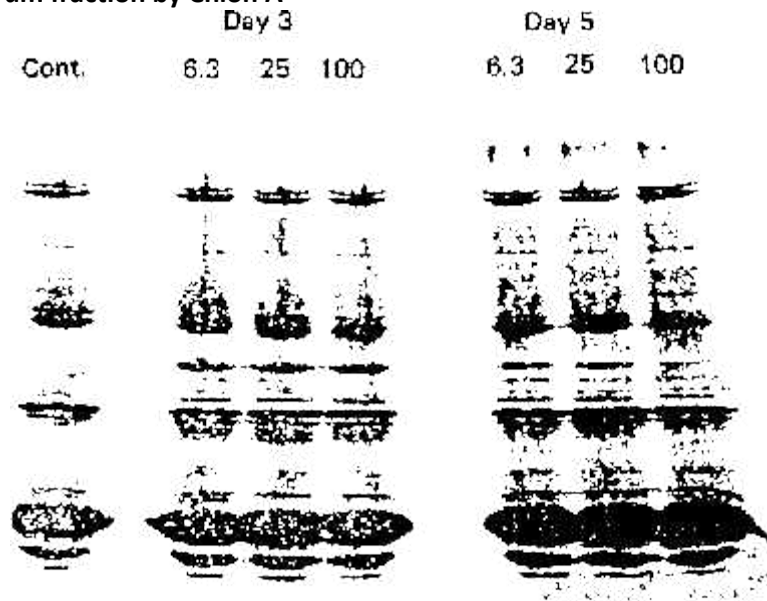


Fig. 2 Effect of Chlon A on the cytolytic activity of peritoneal exudate cells

Fig. 3 Effect of Chlon A on the cytostatic activity of peritoneal exudate cells

Fig. 4 Change of serum fraction by Chlon A



Gel: 4-30% gradient (polyacryl amide)

Buffer: 0.025M Tris-0.19M glycine 10 mA, 17 hrs.