Therapeutic efficacy of Gc protein-derived macrophage activating factor (GcMAF) for adenocarcinoma.

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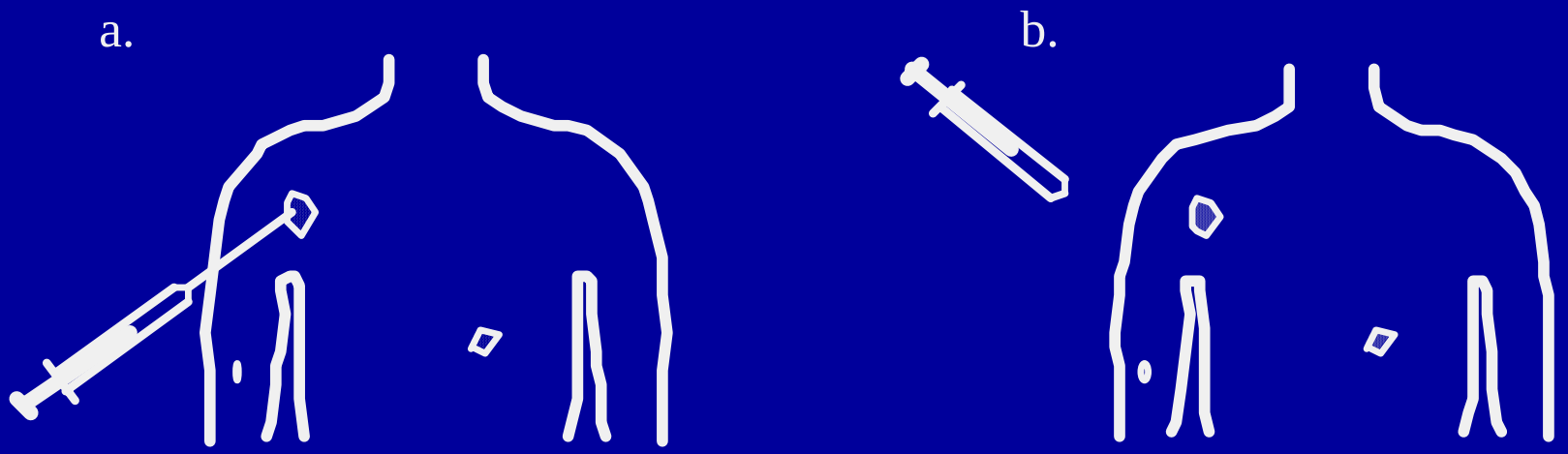
Inflammation in Cancerous and Noncancerous Tissues

Inflammation Induced by Administration of BCG



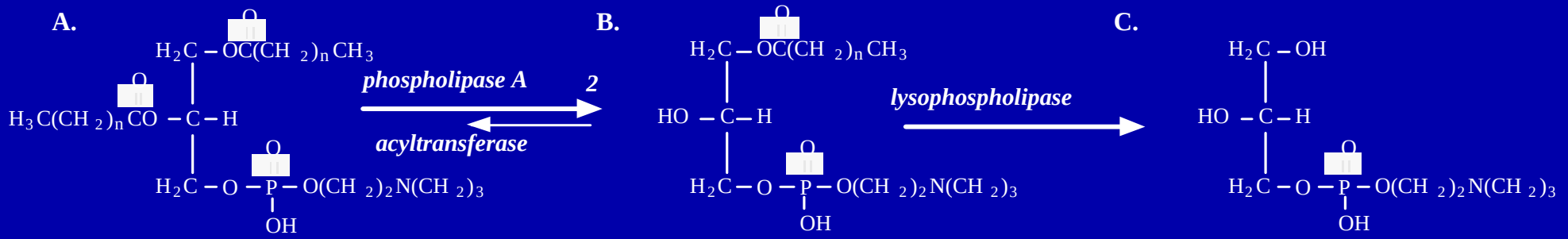
a. Intratumor administration of BCG results in eradication of local as well as metastasized tumors, indicating development of immunity against the tumors.

b. Administration of BCG to noncancerous tissues results in no significant effect on tumors.

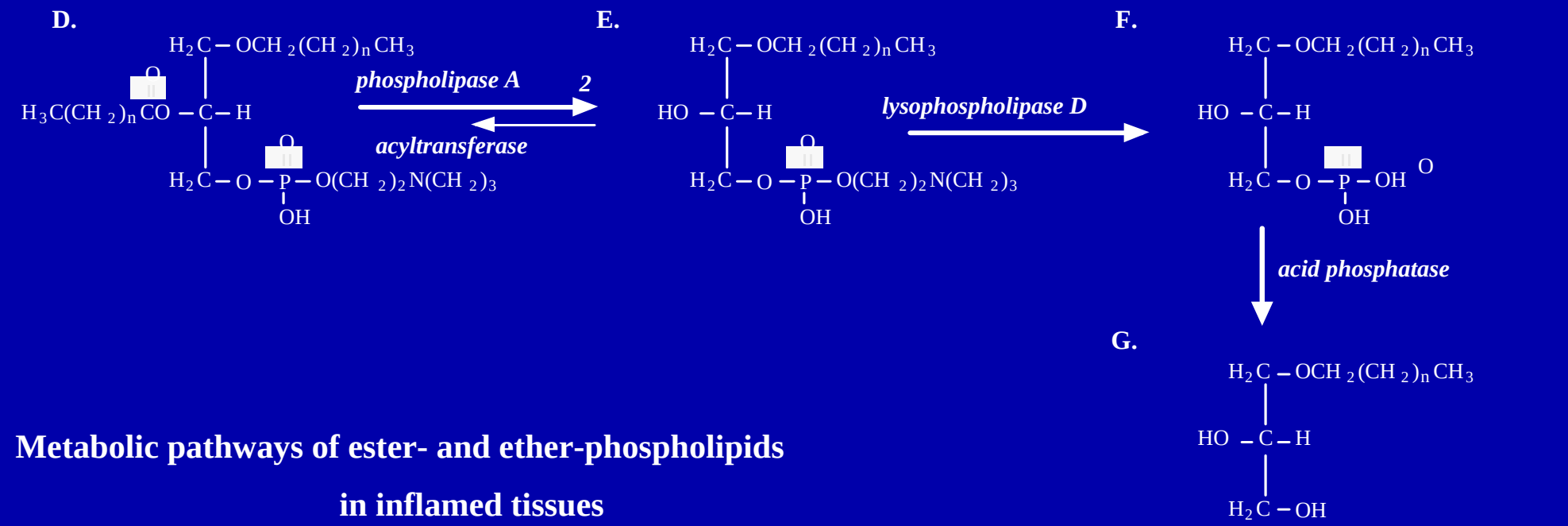
Refs: 1. Morton, D. et al. 1970, Surgery 68: 158-164.

2. Zbar, B. and Tanaka, T. 1971, Science 172: 271-273.

I. Ester phospholipids:



II. Ether phospholipids:



Metabolic pathways of ester- and ether-phospholipids in inflamed tissues

I. Inflammation in noncancerous tissues

1. Phosphatidylcholine (or other Phospholipids)

↓ Phospholipase A_2

2. Lysophosphatidylcholine: (Lyso-Pc ; one of lysophospholipids) is capable of activation of macrophages.

↓ Lysophospholipase

3. Inert compounds

II. Inflammation in cancerous tissues (e.g., administration of BCG)

1. Alkylphospholipids

↓ Phospholipase A_2

2. Lyso-alkylphospholipids: are potent macrophage activating agents

↓ Lysophospholipase D and acid phosphatase

3. Alkylglycerols: are potent macrophage activating agents

Dodecylglycerol (DDG : one of alkylglycerols)

For macrophage activation, DDG is 400 times more active than lyso-Pc

Conclusion: These information suggests that highly activated macrophages can kill and eradicate cancerous cells.

Publications:

1. Yamamoto, N., et al. 1987. Cancer Immuno. Immunother. 25: 185-192.

2. Yamamoto, N. and Ngwenya, B. Z. 1987. Cancer Res. 47: 2008-2013.

3. Yamamoto, N., St. Claire, D. A., Homma, S., and Ngwenya, B. Z. 1988. Cancer Res. 48:6044-6049.

4. Homma, S. and Yamamoto, N. 1990. Clin. Exp. Immunol. 79: 307-313.

I. *In vivo:* Administration of lyso-Pc (20 μg) or DDG (50ng) into mice

activates macrophages

II. *In vitro:* Cultivation of [Macrophages] + lyso -Pc or DDG

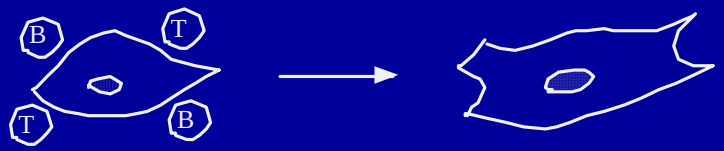
lyso-Pc or DDG

no macrophage activation

macrophages alone

III. *In vitro:* Cultivation of [Macarophages + lymphocytes] + lyso -Pc or DDG

lyso-Pc or DDG

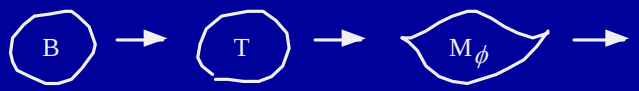


macrophages + lymphocytes

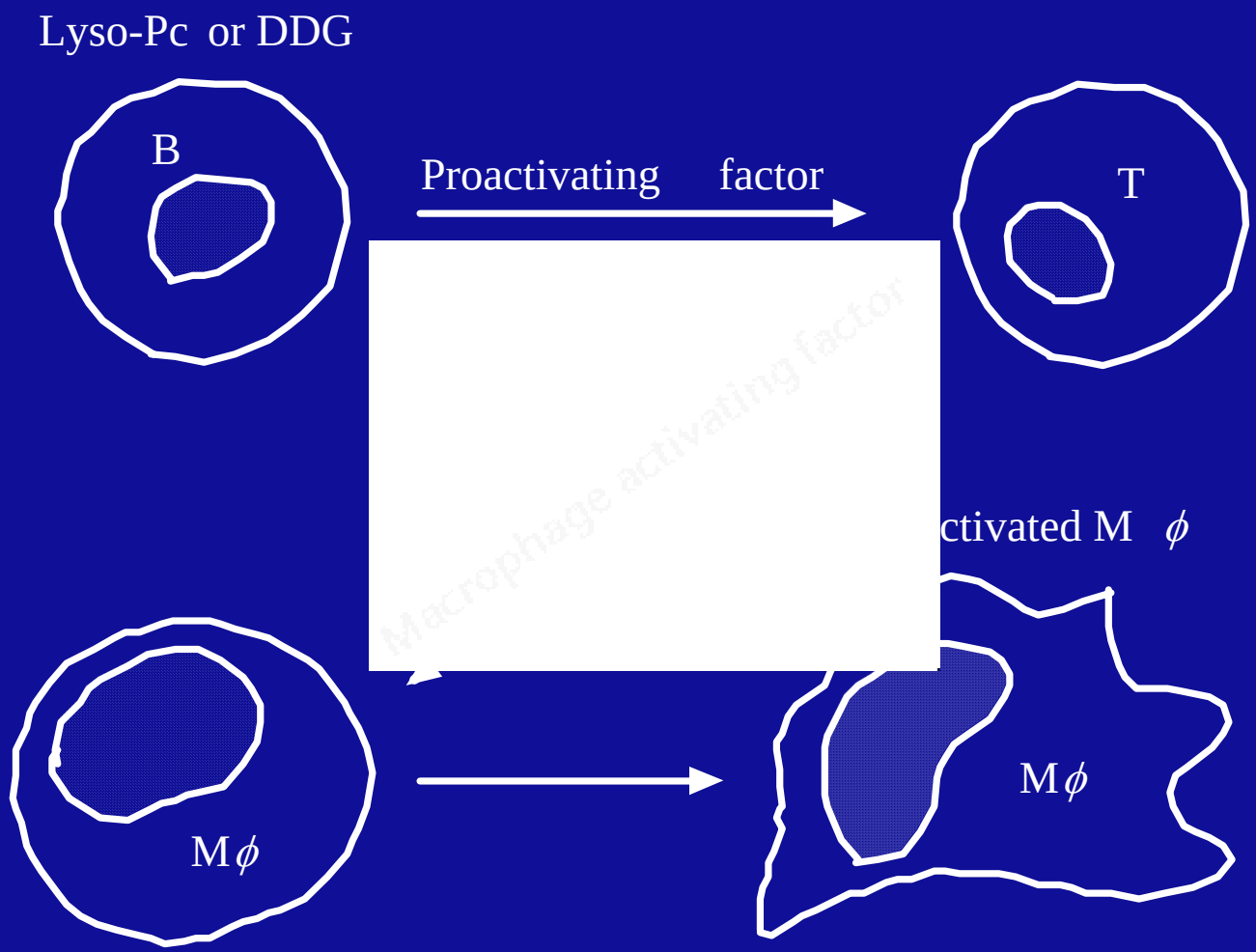
activation

IV. Macrophage activation signaling pathway

lyso-Pc or DDG



activation

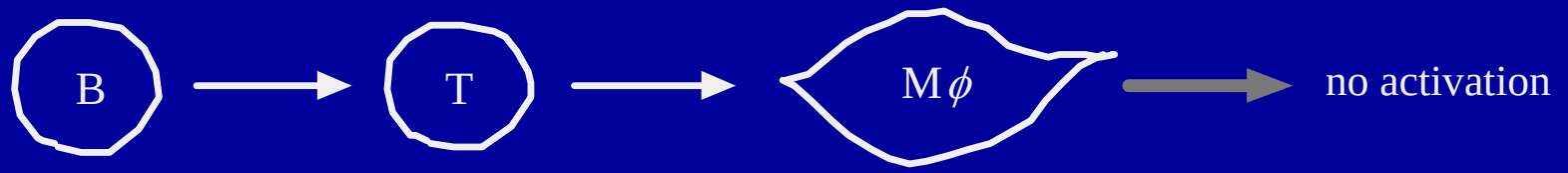


Inflammation primed macrophage activation requires participation of B and T cells.

To search for macromolecular factor to activate macrophages
(macrophage activating factor)

1. In serum free medium

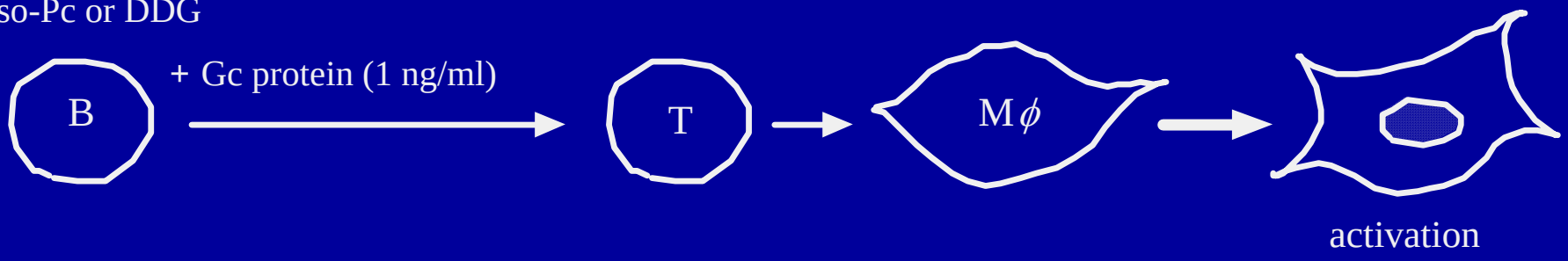
lyso-Pc or DDG



Suggesting macrophage activation requires at least one serum component.

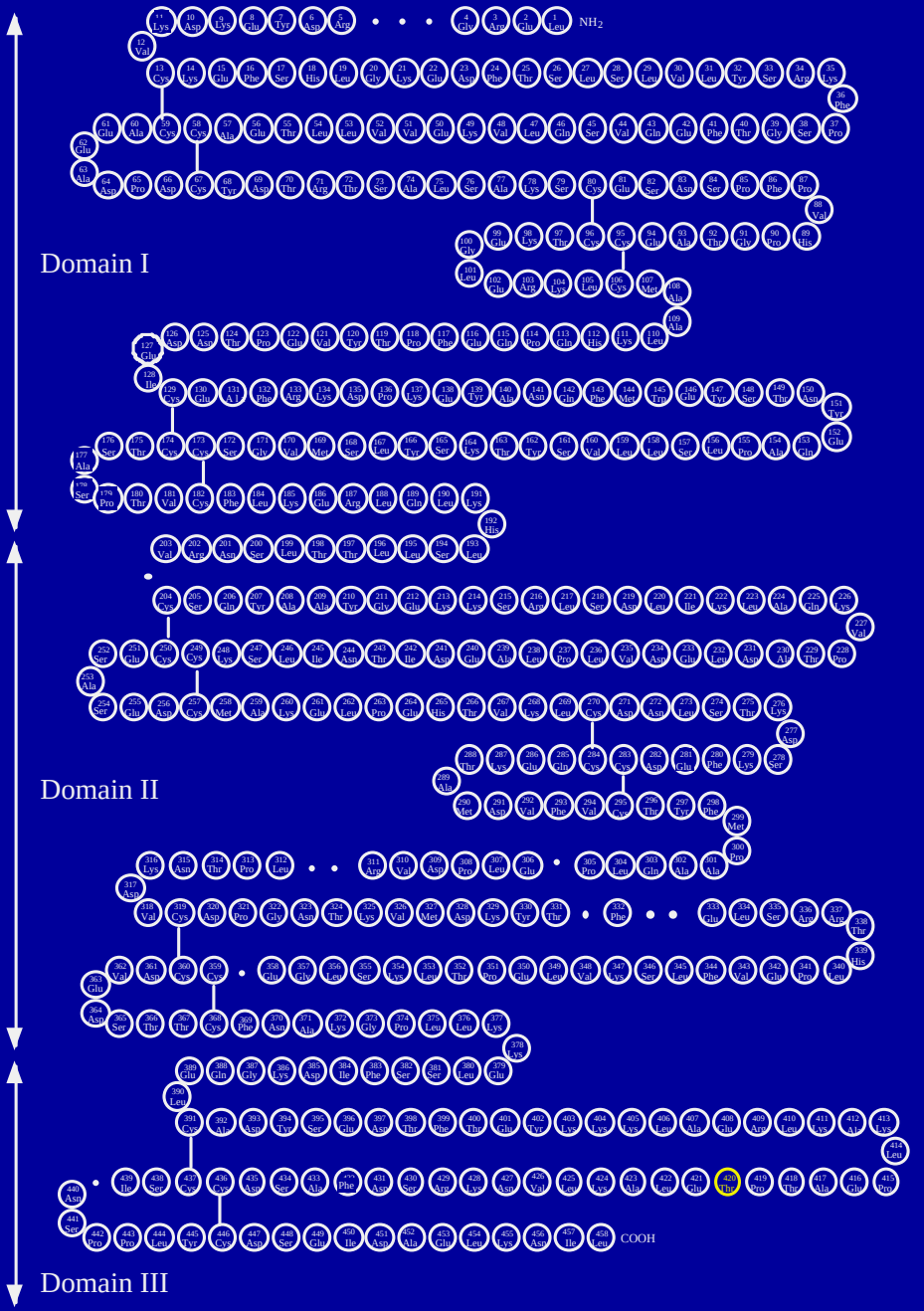
2. Macrophage activation requires serum vitamin D binding proteins (known as Gc protein)

lyso-Pc or DDG

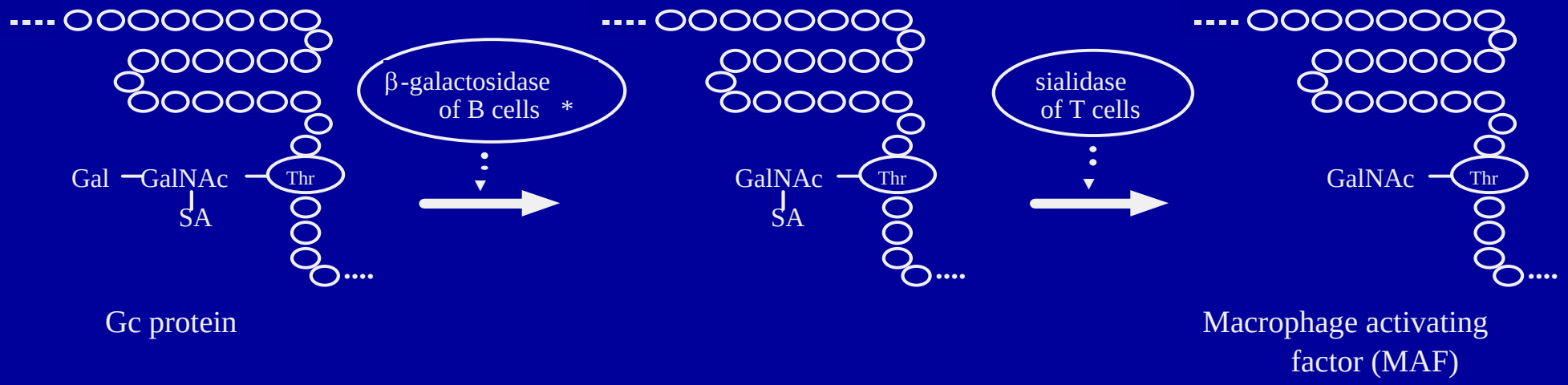


Publications:

1. Yamamoto, N., Homma, S. and Millman, I. 1991. J. Immunol. 147: 273-280.
2. Yamamoto, N. and Homma, S. 1991. Proc. Natl. Acad. Sci. USA 88: 8539-8543.
3. Yamamoto, N., Kumashiro, R., Yamamoto, M., Willett, N. P. and Lindsay, D. D. 1993. Infect. Imm. 61: 5388-5391.
4. Yamamoto, N. and Kumashiro, R. 1993. J. Immunol. 151: 2794-2902.
5. Yamamoto, N., Willett, N. P. and Lindsay, D. D. 1994. Inflammation 18: 311-322.
6. Yamamoto, N., Naraparaju, V. R. and Asbell, S. O.1996. Cancer Res. 56: 2827-2931.
7. Yamamoto, N. 1996. Molecular Immunol. 33: 1157-1164.
8. Yamamoto, N. and Naraparaju, V. R. 1997. Cancer Res. 57: 2187-2192.
9. Koga, Y., Naraparaju, V. R. and Yamamoto, N. 1999. Proc. Soc. Exp. Biol. Med. 220: 20-26.

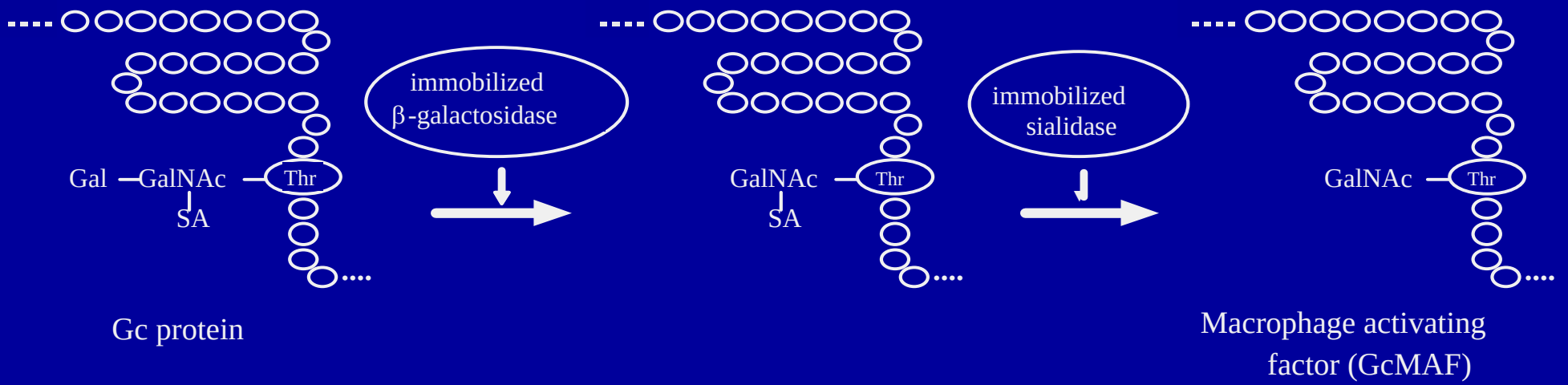


Vitamin D-binding protein (known as Gc protein)



Gc protein

Macrophage activating factor (MAF)

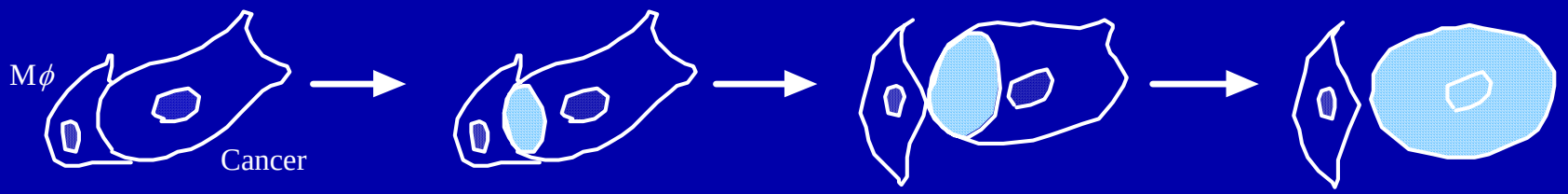


Gc protein

Macrophage activating factor (GcMAF)

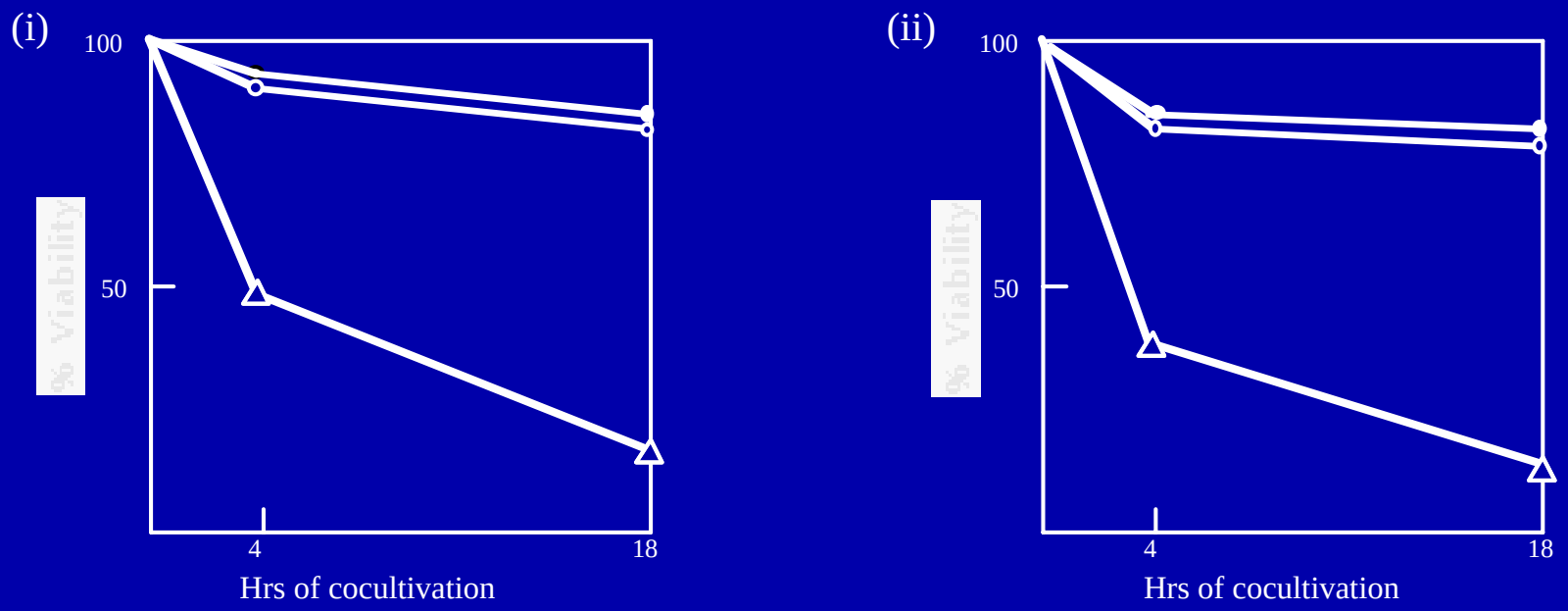
In vitro Tumoricidal Capacity of Macrophages Activated by GcMAF

a. Time course observation of tumoricidal process with trypan blue penetration.



Macrophages were activated by preincubation with GcMAF (100pg/ml) for 3hr.

b. Tumoricidal capacity of GcMAF-treated human macrophages for prostate cancer cells LNCaP (i) and breast cancer cells MDA-MB-231 (ii)



●, tumor cells only; ○, tumor cells with macrophages; △, tumor cells with activated macrophages

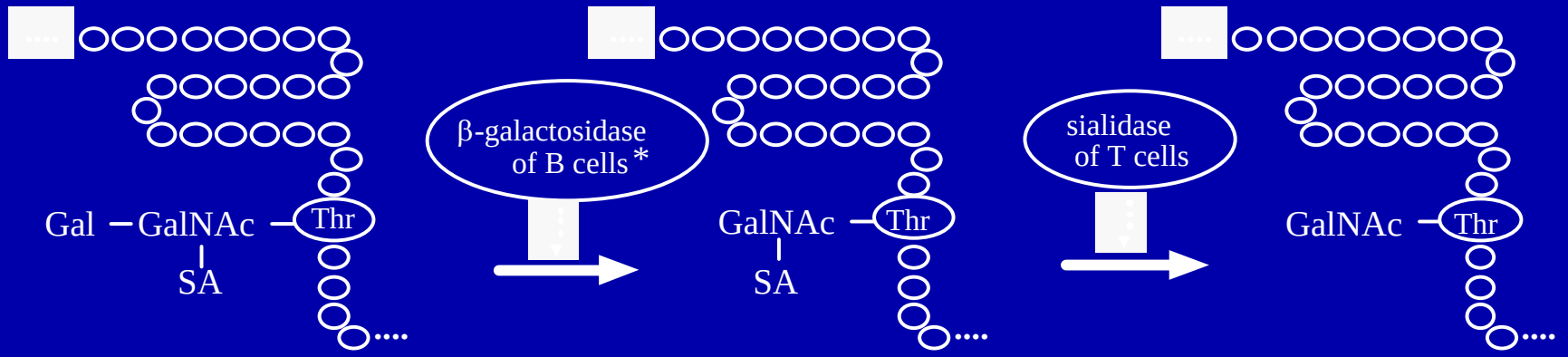
Approximately 3.5 x 10^5 tumor cells were cultured with 1.1 x 10^6 macrophages/well.

Characterization of monocytes/macrophages, lymphocytes and Serum Gc protein of individual oral cancer patients.

Patient No.	Assayed on:	nmole superoxide produced/min/10^6 phagocytes			
		phagocytes*		lymphocytes/phagocytes**	
		none	100pg GcMAF	1ng Gc protein	0.1% serum
1		0.21	6.38	5.87	2.61
2		0.66	5.99	6.03	1.42
3		0.71	6.34	5.65	5.19
4		0.40	5.96	5.72	1.07
5		0.62	6.83	5.64	3.45
6		0.56	5.92	5.87	1.11
7		0.33	5.84	5.94	0.72
8		0.72	5.96	5.63	1.19
9		0.29	6.70	6.26	2.14
10		0.62	6.34	6.08	0.31
Healthy humans		0.27	6.68	6.22	4.25

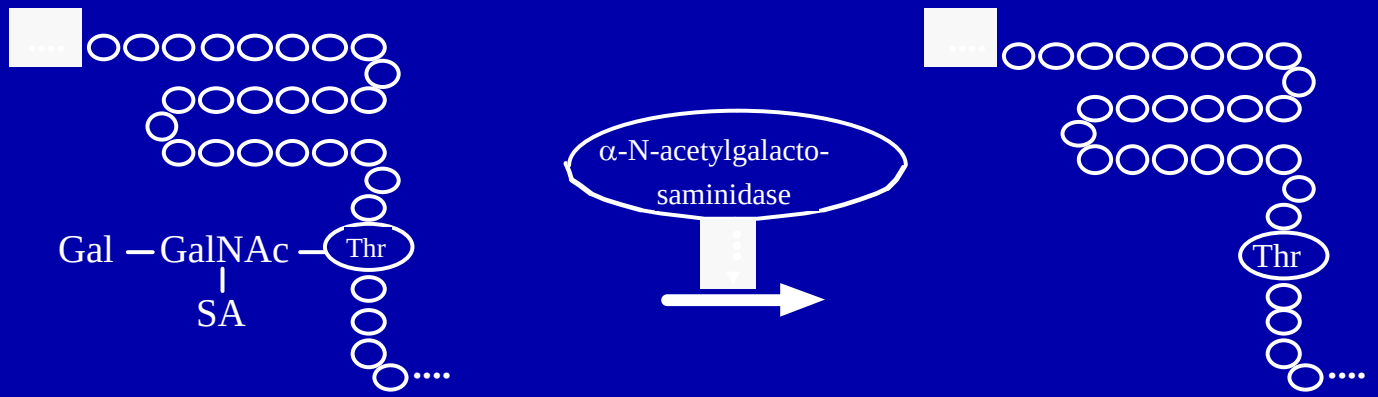
* lyso-Pc untreated. ** lyso-Pc-treated lymphocytes + phagocytes.

Precursor activity of serum Gc protein were analyzed using 0.1% patient serum.



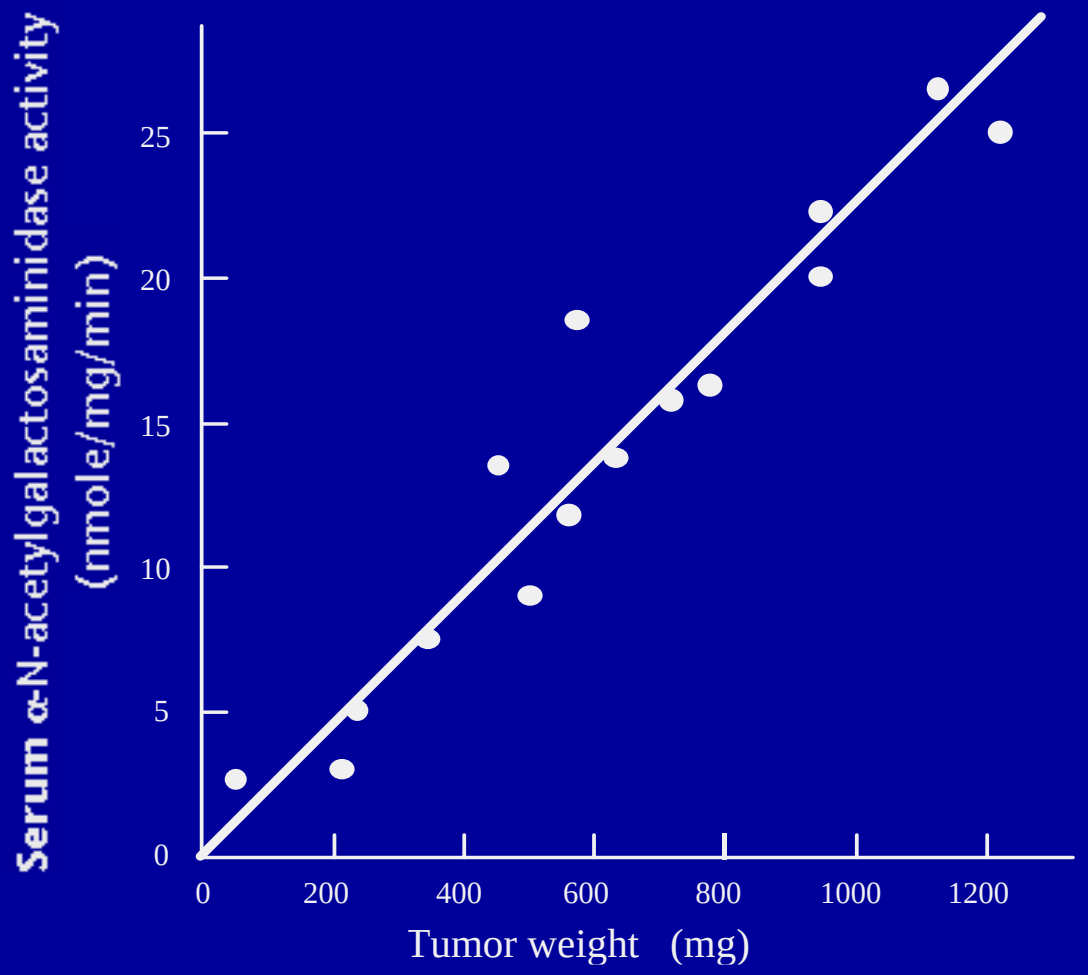
Gc protein

Macrophage activating factor (MAF)



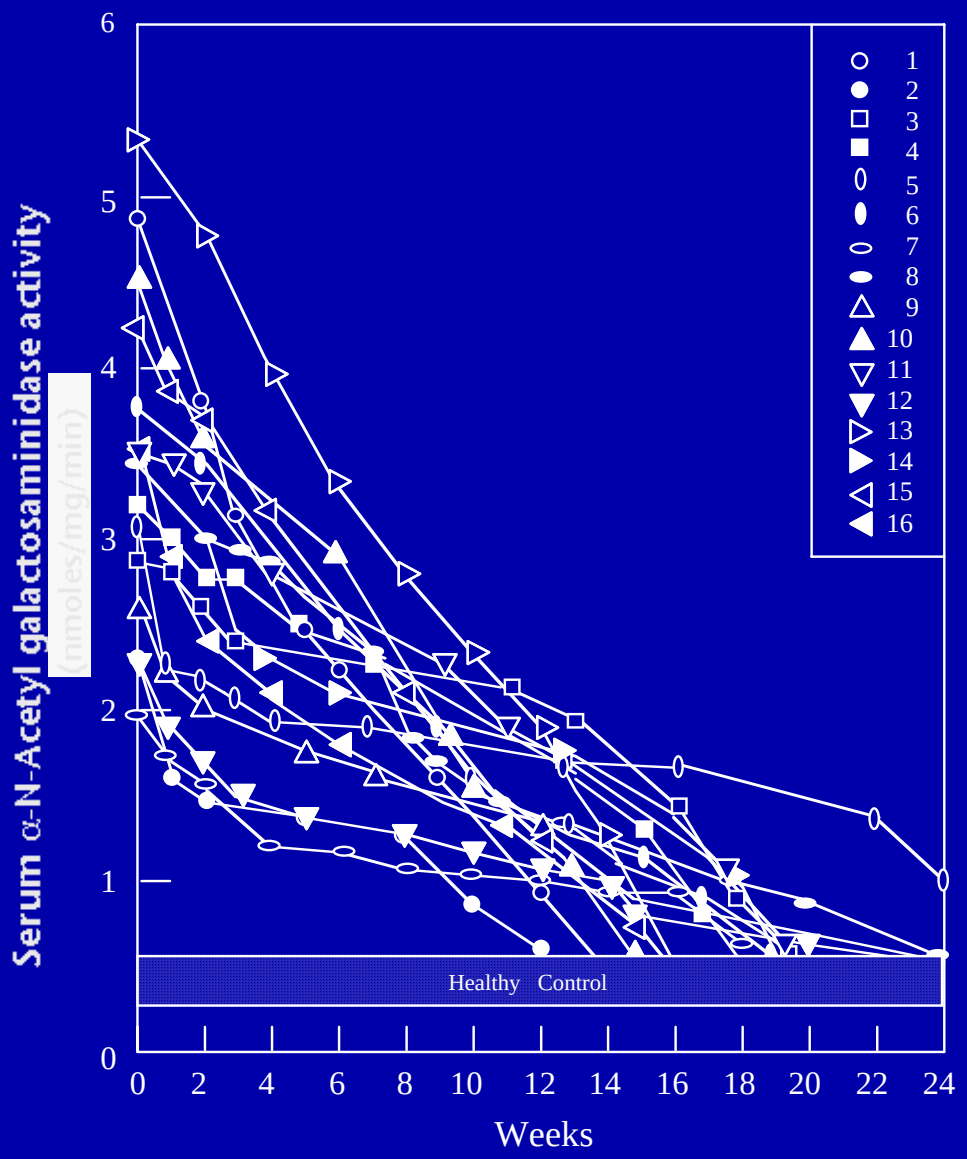
Gc protein

Deglycosylated Gc protein



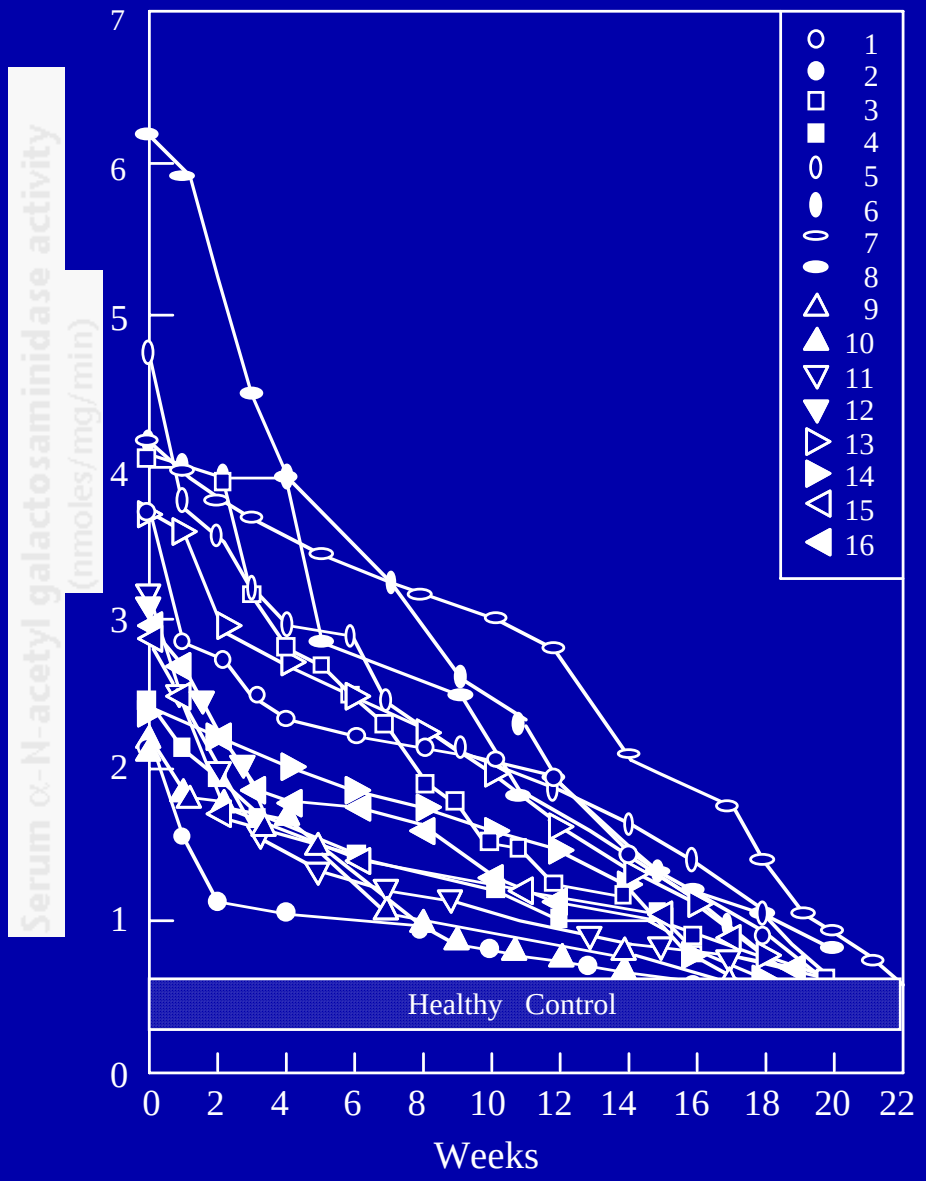
Correlation between serum α-N-acetylgalactosaminidase activity and tumor burden (measured by total weight) in nude mouse transplanted with human oral squamous cell carcinoma (KB) cell line.

GcMAF therapy for prostate cancer patients



Weekly administration of 100ng GcMAF

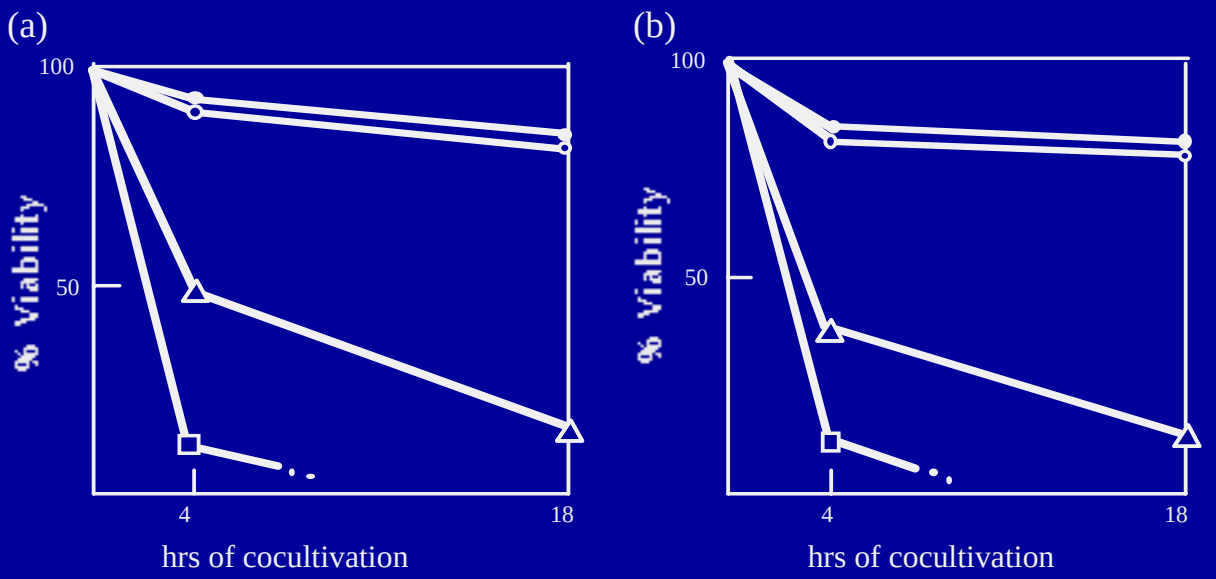
GcMAF therapy for breast cancer patients



Weekly administration of 100ng GcMAF

In vitro Tumoricidal Capacity of Macrophages Activated by GcMAF

Tumoricidal capacity of GcMAF-treated human macrophages for prostate cancer cells LNCaP (a) and breast cancer cells MDA-MB-231 (b).



● , tumor cells only;
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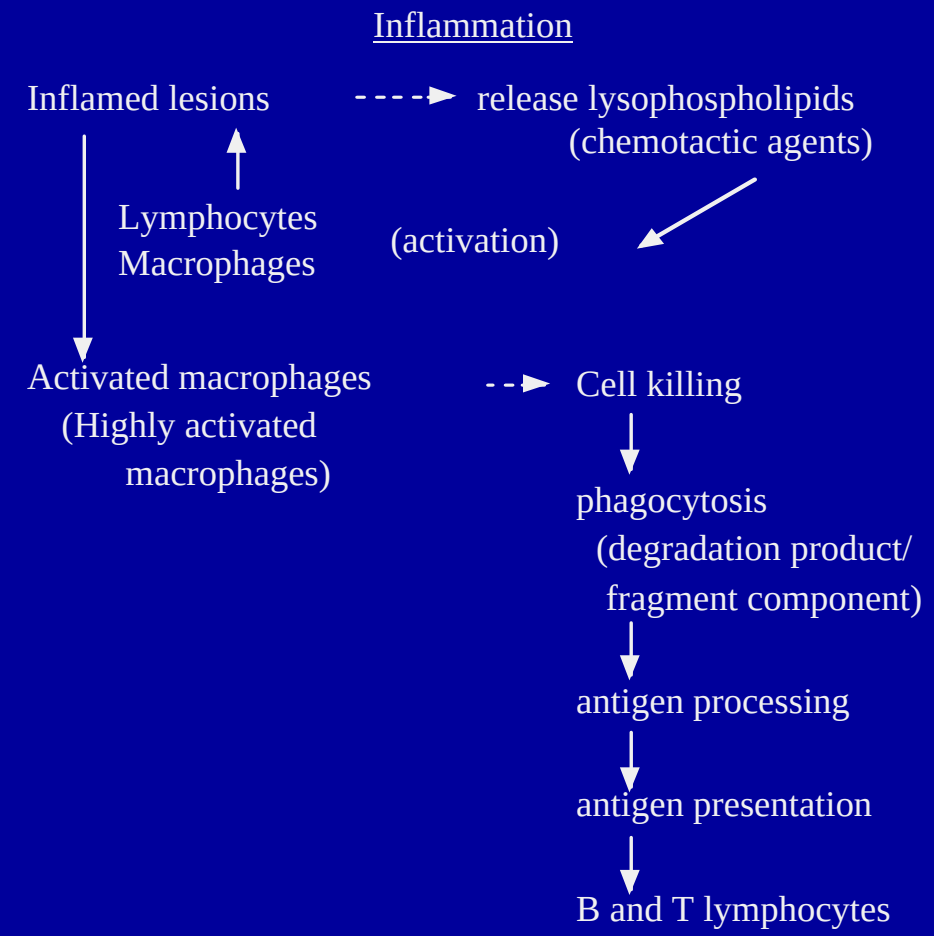
△, tumor cells with activated macrophages;
□ , patient serum coated cancer cells with activated macrophages.

Approximately 3.5 x 10^{5} tumor cells were cultured with 1.1 x 10^{6} macrophages/well. Time course observation of tumoricidal process was performed by trypan blue exclusion vital assay.

IMMUNE DEVELOPMENT

Principal Immune Development Cascade



Development of Humoral and Cellular Immunity*

*Macrophage activation is the first mandatory step for immune development. Thus, lack of macrophage activation leads to immunosuppression.