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

Nobuto Yamamoto, Masahiro Urade, and Masumi Ueda. Socrates Institute for Therapeutic Immunology, Philadelphia, PA and Hyogo College of Medicine, Hyogo, Japan.
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.

I. Ester phospholipids:

A. \[ \text{phospholipase A} \]

\[ \begin{align*} 
H_2C & - OC(CH_2)_nCH_3 \\
H_3C(CH_2)_nCO & - C - H \\
H_2C & - O - P - O(CH_2)_2N(CH_2)_3 \\
\text{acyltransferase} &
\end{align*} \]

B. \[ \text{lysophospholipase} \]

\[ \begin{align*} 
H_2C & - OCH_2(CH_2)_nCH_3 \\
H_3C(CH_2)_nCO & - C - H \\
H_2C & - O - P - O(CH_2)_2N(CH_2)_3 \\
\text{acyltransferase} &
\end{align*} \]

II. Ether phospholipids:

D. \[ \text{phospholipase A} \]

\[ \begin{align*} 
H_2C & - OCH_2(CH_2)_nCH_3 \\
H_3C(CH_2)_nCO & - C - H \\
H_2C & - O - P - O(CH_2)_2N(CH_2)_3 \\
\text{acyltransferase} &
\end{align*} \]

F. \[ \text{acid phosphatase} \]

\[ \begin{align*} 
H_2C & - OCH_2(CH_2)_nCH_3 \\
H_3C(CH_2)_nCO & - C - H \\
H_2C & - O - P - OH \\
\text{acyltransferase} &
\end{align*} \]

Metabolic pathways of ester- and ether-phospholipids

in inflamed tissues
I. Inflammation in noncancerous tissues

1. Phosphatidylcholine (or other Phospholipids)
   - Phospholipase A
   - Lysophospholipase

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. 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:


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

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

   lyso-Pc or DDG

   no macrophage activation

   macrophages alone

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

   lyso-Pc or DDG

   macrophages + lymphocytes

   activation

IV. Macrophage activation signaling pathway

   lyso-Pc or DDG

   B → T → Mφ 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

B + Gc protein (1 ng/ml)

activation
Publications:

Vitamin D-binding protein (known as Gc protein)
Gal → GalNAc → Thr

β-galactosidase of B cells

GalNAc → Thr

Sialidase of T cells

GalNAc → Thr

Macrophage activating factor (MAF)

Gal → GalNAc → Thr

Immobilized β-galactosidase

GalNAc → Thr

Immobilized sialidase

GalNAc → Thr

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)

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.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Assayed on:</th>
<th>nmole superoxide produced/min/10^6</th>
<th>6 phagocytes</th>
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<tr>
<td></td>
<td></td>
<td>phagocytes*</td>
<td>lymphocytes/phagocytes**</td>
</tr>
<tr>
<td></td>
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<td>100pg GcMAF</td>
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<td>Healthy humans</td>
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</table>

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

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

Gal → GalNAc → Thr
SA

Macrophage activating factor (MAF)

β-galactosidase of B cells *

GalNAc → Thr
SA

α-N-acetylgalactosaminidase

GalNAc → Thr

Deglycosylated Gc protein

Gal → GalNAc → Thr
SA
Correlation between serum $\alpha$-N-acetyl-

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

Approximately $3.5 \times 10^5$ tumor cells were cultured with $1.1 \times 10^6$ macrophages/well. Time course observation of tumoricidal process was performed by trypan blue exclusion vital assay.
IMMUNE DEVELOPMENT
Principal Immune Development Cascade

Inflammation

- - - - - release lysophospholipids (chemotactic agents)

- - - - - - - - (activation)

- - - - - Cell killing

- - - - - phagocytosis (degradation product/fragment component)

- - - - - antigen processing

- - - - - antigen presentation

- - - - B and T lymphocytes

Development of Humoral and Cellular Immunity*

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