

論 文 内 容 要 旨

報告番号	甲 先 第 244 号	氏 名	口池 大輔
学位論文題目	Degalactosylated/Desialylated Human Serum Containing GcMAF Induces Macrophage Phagocytic Activity and <i>In Vivo</i> Antitumor Activity (GcMAF 含有ヒト血清のマクロファージ食食活性化能評価方法の開発)		
<p>内容要旨</p> <p><i>Background:</i></p> <p>Macrophages are known to have a critical role in antitumor immunity, can infiltrate into tumors and are found in most tumor sites. Macrophage also has a function as an effector of cell immunity via presentation of an antigen to T cell and production of interleukin 1. Accordingly, it is important to activate macrophage for treatment and prevention of a cancer and an infectious disease, and the activation of macrophage makes it possible to carry out treatment and prevention of a cancer and an infectious disease.</p> <p>A factor for activating macrophage is, for example, an interferon, and its clinical application has been carried out. In addition, it is known that a certain kind of polysaccharides has an immunostimulating activity, and some of them are expected to be developed as an antiviral agent and an anticancer agent.</p> <p>The group-specific component (Gc) protein-derived macrophage-activating factor (GcMAF) has various biological activities such as macrophage activation and antitumor activity. Clinical trials of GcMAF have been carried out for metastatic breast cancer, prostate cancer, and metastatic colorectal cancer. Saisei Mirai will have treated more than 1,000 patients with GcMAF-containig human serum, both with and without conventional therapies.</p> <p>Thus, we propose a novel method for preparation of autologous serum containing GcMAF by degalactosylation and desialylation and report the potential role of autologous GcMAF in stimulating</p>			

phagocytosis in macrophages and *in vivo* antitumor activity.

Colostrum is a type of milk produced by the mammary glands of mammals just prior to giving birth. It contains serum proteins and antibodies such as albumin, insulin-like growth factor (IGF), epidermal growth factor (EGF), nerve growth factor (NGF), lactoferrin, immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) to protect the newborn against various infectious diseases. In this study, we hypothesized that colostrum could be a macrophage-activator if enzymatically modified IgA and Gc protein had activity similar to that of GcMAF. Therefore, we propose a novel macrophage-activator function for degalactosylated/desialylated bovine colostrum and report the potential role of this modified bovine colostrum in stimulating phagocytosis in macrophages *in vitro* and *in vivo*.

Materials and methods:

Gc protein and GcMAF were prepared from human serum containing 1f1f-subtype of Gc protein obtained from human volunteers and colostrum MAF was prepared from bovine colostrum obtained from Jun Sei Co. Ltd. (Tokyo, Japan). Each sample was incubated with β -D-galactosidase and neuraminidase. The reaction mixture was heated and concentrated. The protein concentrations were determined using a BCA protein assay kit. The GcMAF-containing human serum and colostrum MAF were diluted adequately by using saline solution.

They were subjected to sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and subsequently electroblotted onto a nitrocellulose membrane. The blots were probed with biotin-conjugated *Helix pomatia* agglutinin (HPA) lectin specific for GcMAF with *N*-acetylgalactosamine (GalNAc) moiety and incubated with horseradish peroxidase (HRP)-labeled anti-rabbit IgG as a secondary antibody to achieve with visualization and quantification of the western blot bands.

Mouse peritoneal macrophages for phagocytosis assay were collected from 8-week-old female ICR mice. The cells were treated with GcMAF-containing human serum and colostrum MAF, the cultures were assayed for phagocytic activity with opsonized sheep red blood cells. The number of phagocytosed erythrocytes per cell was determined microscopically; 250 macrophages were counted for each data point. The data were expressed

in terms of the phagocytosis index (PI), which was defined as the percentage of macrophages with ingested erythrocytes multiplied by the mean number of erythrocytes ingested per macrophage.

In vivo antitumor activity assay was measured with Ehrlich ascites carcinoma (EAC) carrying mouse. Ten-week old female ICR mice were inoculated with 1×10^7 EAC cells intraperitoneally and administered intraperitoneally with GcMAF-containing human serum for 7 days. The animals with ascitic tumor were weighed every day. Kaplan-Meier survival curves were analyzed by the log-rank test. All procedures used for animal experimentation were approved by the Animal Research Committee of the University of Tokushima (TokuDobutsu12025).

Data are expressed as mean and standard deviation. The statistical significance of the differences between the results of the independent experiments was analyzed using Student's *t* test. A *P* value of <0.05 was considered statistically significant.

Results:

We detected GcMAF in degalactosylated/desialylated human serum and colostrum MAF in degalactosylated/desialylated bovine colostrum by western blotting using anti-human Gc globulin antibody, and *Helix pomatia* agglutinin lectin. We also found that GcMAF-containing human serum enhance the phagocytic activity of mouse peritoneal macrophages and extend the survival time of mice bearing Ehrlich ascites tumor. And, we also evaluated colostrum MAF for its ability to activate mouse peritoneal and intestinal macrophage phagocytosis *in vitro* and *in vivo*.

Conclusion:

We propose that GcMAF-containing human serum and degalactosylated/desialylated bovine colostrum can be used as an effective phagocytosis activator for macrophages that does not induce inflammatory cytokines and antitumor agent for cancer immunotherapy.

論文審査の結果の要旨

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学位論文題目 Degalactosylated/Desialylated Human Serum Containing GcMAF Induces Macrophage Phagocytic Activity and <i>In Vivo</i> Antitumor Activity (GcMAF含有ヒト血清のマクロファージ貪食活性化能評価方法の開発)			
審査結果の要旨 自然免疫の主要なプレーヤーであるマクロファージに着目し、患者自身の血清を用いて直接GcMAFを調製してそれを患者に投与する治療法を開発するため、健常人の血清を用いたGcMAF調製と得られたGcMAF含有ヒト血清のマクロファージ貪食活性化能および <i>in vivo</i> 抗腫瘍活性について調査した。その結果、GcMAF含有ヒト血清は未処理血清に比べてGalNAc特異的HPAレクチンに対して陽性反応を示す3つのバンドが存在し、55 KDa付近のバンドがGcMAFと一致したことから血清中のGcMAFの生成が確認された。また、GcMAF含有ヒト血清は1 ng~1 µgの範囲でコントロールよりも有意に高いマクロファージ貪食活性化を示した。一方、未処理血清は貪食活性化能を示さなかった。さらに、1.55 mg/kgのGcMAF含有ヒト血清を連日投与したマウスは、未処理群に比べて有意に生存期間が延長し、中央値24.5日（コントロール：17.8日）、延長率1			

38%を示した。この結果より、GcMAF含有ヒト血清はGcMAFと同様にマクロファージ食食活性化能と *in vivo* 抗腫瘍活性を有することが示された。

次に、抗体や成長因子などの血清タンパク質を含有することが知られているウシ初乳を用いて、経口摂取が可能なマクロファージ活性化剤の開発を試みた。ウシ初乳MAFは、未処理ウシ初乳と比べて有意なHPAレクチン陽性反応を示し、 β -ガラクトシダーゼのみで処理した場合は75 KDa付近に陽性バンドが、 β -ガラクトシダーゼとシアリダーゼで処理した場合は180, 75, 30 KDa付近により強い陽性バンドが観察された。また、 β -ガラクトシダーゼ単独もしくはシアリダーゼで併用処理したウシ初乳MAFは、ウシ初乳と比べて有意なマクロファージ食食活性化能を示し、その活性値はLPSやGcMAFと同程度であった。以上の結果より、ウシ初乳タンパク質を酵素処理して調製した初乳MAFは、血清糖タンパク質GcMAFと同程度の強いマクロファージ食食活性化能を有することが示された。

以上本研究は、ヒト血清やウシ初乳を利用したマクロファージ活性化剤の創製に関する研究であり、本論文は博士（工学）の学位授与に値するものと判定する。