CHARACTERIZATION OF HUMAN Gc PROTEIN-DERIVED MACROPHAGE ACTIVATION FACTOR (GcMAF) AND ITS FUNCTIONAL ROLE IN MACROPHAGE TUMORICIDAL ACTIVITY

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1. INTRODUCTION

Macrophages are essential for host defense and play an important role in orchestrating immune response of the host against threat signals. Macrophages are also known to have a critical role in antitumor immunity,[1] can infiltrate into tumor, and are found in most tumor sites.[2,3] Meanwhile, Gc protein (also known as vitamin D_3-binding protein) is a serum protein with multifunctional properties[4] and has been reported as a precursor for macrophage activation factor.[5] Gc protein can be converted by an inducible ß-galactosidase of B cells and neuraminidase of T cells to a potent macrophage activating factor (GcMAF), a protein with *N*-acetylgalactosamine (GalNAc) as the remaining sugar moiety.[6,7] Activated macrophages express tumoricidal activity by ingestion of tumor cells and release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), or both.[5] We reported that *in situ* modification of Gc protein with ß-galactosidase and neuraminidase increased the release of superoxide in thioglycolate-elucidated mouse peritoneal macrophage.[8] Yamamoto et al. reported the possibility of using GcMAF as an immunomodulator for cancer treatment,[9] so it is important to provide an assay for GcMAF. Kanan et al. reported the quantitative analysis of GcMAF from human serum.[10] However, the sugar moiety of GcMAF has never been qualitatively studied. Here we present the study of qualitative analysis of GcMAF from purified human serum as well as its influence on the macrophage activity.

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2. MATERIALS AND METHODS

2.1. Materials

25-OH-Vitamin D_3 was a gift from Dr. Nobuto Yamamoto from Socrates Institute for Therapeutic Immunology and Albert Einstein Cancer Center, Philadelphia, USA. Other chemicals (biochemical grade) were purchased from Wako Pure Chemical Industries Co., Japan.

2.2. Purification of human serum Gc protein

The procedure was adapted from methods reported by Link et al.[11]

Human serum was diluted 1:1 with column buffer and applied to the 25-OH-D_3-Sepharose column at a rate of 0.4 mL/min. Absorbance at 280 nm was used to monitor protein elution from the column. After the sample was applied, the column was washed with 300 mL of column buffer at 2 mL/min. The protein remaining on the matrix was eluted with 6 M guanidine-HCl at 1 mL/min and 1 mL/fraction was collected. Fractions with protein peak of the guanidine eluted fraction were pooled and dialyzed with 10 mM sodium phosphate, pH 7.0 for the hydroxyappatite chromatography.

2.2.2. Hydroxyappatite chromatography

A 5-mL hydroxyappatite column (Econo-Pac HTP Cartridge 1, Bio-Rad) was equilibrated in 10 mM sodium phosphate, pH 7.0. The dialyzed sample from 25-OH-D_3-Sepharose chromatography was applied to the column (0.5 mL/min) and the column was washed with 50 mL 10 mM sodium phosphate, pH 7.0 (2 mL/min). A linear gradient from 10 mM sodium phosphate to 200 mM sodium phosphate, pH 7.0 was applied to the column. Fractions with protein peak were collected and the protein concentration was determined using the BCA method. Collected fractions were stored at -80 °C.

2.3. GcMAF preparation

Purified Gc protein (100 μg) was incubated with immobilized ß-galactosidase (1 Unit, in 0.5 mL 100 mM sodium phosphate buffer, pH 7.0) in a microcentrifuge tube at 37 °C by rotation movement for 1 hr. The immobilized enzyme was removed by centrifugation and pH of the supernatant was adjusted to pH 6.0 using 1M NaH_2PO_4. The supernatant (1.5~2.0 mL in 100 mM sodium phosphate buffer, pH 6.0) was incubated with immobilized neuraminidase (0.5 Unit) in a microcentrifuge tube at 37 °C by rotation movement for 1 hr. The immobilized enzyme was removed by centrifugation and the supernatant was made sterile by filtration and protein concentration was determined using the BCA method and then stored at -80 °C.