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Stability of GcMAF in serum

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Purpose

We evaluated the stability of GcMAF in serum under room temperature and 40 degrees by the macrophage phagocytic activity of GcMAF in order to confirm whether long-term transportation of GcMAF is possible.

Materials & Methods

GcMAF: β-galactosidase-treated 1f1f subtype human serum (SY-2-78-1). This sample was stored at 4 degrees for 1 year.

Isolation and culture of mouse peritoneal macrophages: Resident mouse peritoneal macrophages were collected from 8-week-old female Institute for Cancer Research (ICR) mice. After centrifugation at 1,000 × rpm at 4°C for 15 minutes, the collected macrophages were cultured in 24-well plates with a density of 5 × 105 cells/well in serum-free RPMI 1640 for 1 hour. The cultured cells were then washed 3 times with serum-free RPMI 1640 to separate adherent macrophages from non-adherent cells such as T and B cells. The cultured macrophages were then treated as indicated for 15 hours, and the phagocytosis assay was performed as described below.

Phagocytosis assay of GcMAF: Mouse peritoneal cells were layered onto coverslips in a 24-well plate. After 3 hours of drug treatment, the cultures were assayed for phagocytic activity. Sheep red blood cells (SRBCs) were opsonized by rabbit hemolytic serum (anti-sheep red blood cells, Cosmo Bio Co., Tokyo, Japan). Opsonized SRBCs (0.5%) in serum-free RPMI 1640 were overlaid on each macrophage-coated coverslip and were cultured for 1.5 hours. The non-internalized erythrocytes were lysed by immersing the coverslip in a hypotonic solution (1/5 phosphate-buffered saline). The macrophages were fixed with methanol, air-dried, and stained with Giemsa stain. The number of phagocytosed erythrocytes per cell was determined microscopically; 250 macrophages were counted for each data point. The data are expressed in terms of the phagocytosis index, which is defined as the percentage of macrophages with ingested erythrocytes multiplied by the average number of erythrocytes ingested per macrophage.

Statistical analysis. Data are expressed as the mean (SD). The significance in the differences between the results of the independent experiments was analyzed using the Student's t test. A P value of <0.05 was considered to be significant.

Results

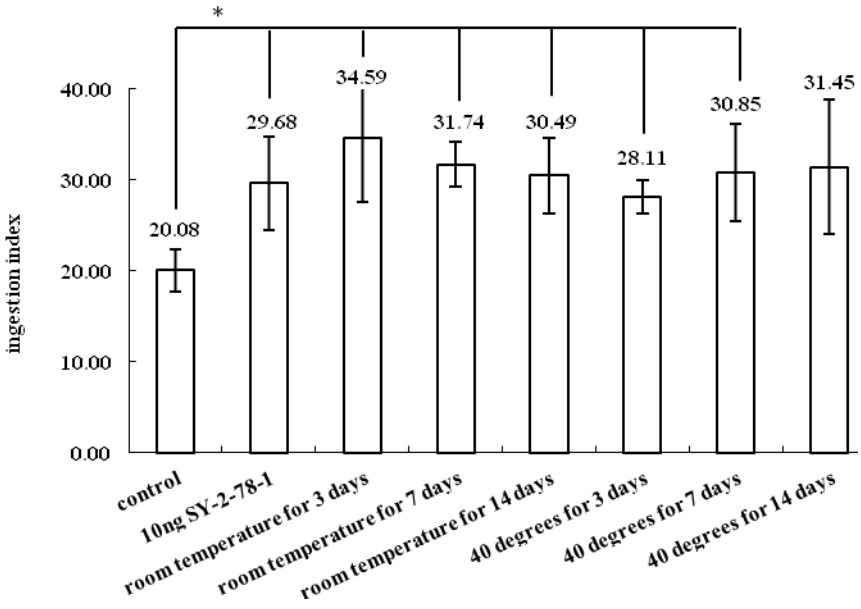

Figure 1 Phagocytic activity of mouse peritoneal macrophage treated with different conditions of GcMAF (room temperature is almost 20 degrees).

Table 1 Ingestion index of mouse peritoneal macrophage treated with GcMAF

	ingestion index			AV	STD	ingestion index /control			ingestion index /control(AV)	STD
control	19.88	17.82	22.55	20.08	2.37	1.00	1.00	1.00	1.00	0.00
10ng SY-2-78-1	30.87	24.01	34.15	29.68	5.17	1.55	1.35	1.51	1.47	0.11
room temperature for 3 days	36.83	26.71	40.24	34.59	7.04	1.85	1.50	1.78	1.71	0.19
room temperature for 7 days	32.37	29.04	33.81	31.74	2.45	1.63	1.63	1.50	1.59	0.07
room temperature for 14 days	29.19	27.20	35.09	30.49	4.10	1.47	1.53	1.56	1.52	0.04
40 degrees for 3 days	28.95	26.02	29.37	28.11	1.82	1.46	1.46	1.30	1.41	0.09
40 degrees for 7 days	32.75	24.86	34.96	30.85	5.31	1.65	1.39	1.55	1.53	0.13
40 degrees for 14 days	28.55	25.96	39.83	31.45	7.37	1.44	1.46	1.77	1.55	0.18

β-galactosidase-treated 1f1f subtype human serum (SY-2-78-1) was showed significant macrophage phagocytic activity on all conditions without 40 degrees for 14 days (Figure 1 and table 1).

Conclusion

GcMAF in serum is stable for 1 year at 4 degrees, for 14 days at room temperature (almost 20 degrees), and for 7 days at 40 degrees.