THP-1 Monocyte Differentiation and Activation

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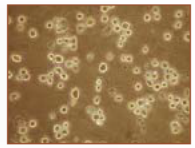


Introduction

Cancer continues to be one of the major causes of death worldwide. According to the American Cancer Society Cancer, 1.5 million cases were reported in 2010. Cancer is characterized by rapid and uncontrolled cell proliferation. Lipid metabolites released from cancerous cell during inflammation are powerful macrophage activating agents. Macrophages are white blood cells from the monocyte lineage residing in tissues that kill pathogensand cancer cells. Serum Gc protein, vitamin D3-binding protein, is the precursor of the macrophage activating factor (Gc-MAF). Conversion of Gc protein to Gc-MAF depends on the action of enzymes on B and T lymphocytes exposed to inflammatory agent. Activated macrophages synthesize and release superoxide anions (O_2). Nagalase released from cancer cells prevents conversion of Gc protein to Gc-MAF, thus leading to immunosuppression[1].

Previous studies have shown mouse macrophage cell line RAW 264 responds to Gc-MAF[2] by secreting O_2 . This experiment is the first to use the human monocytic leukemia cell line THP-1[3] as a model. We hypothesize that differentiation of human monocytes via vitamin D will enhance the production of superoxide anions following Gc-MAF treatment.

Gc-MAF Formation

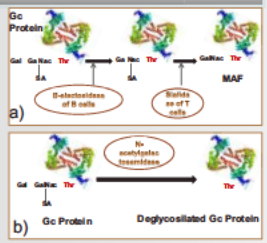


Fig. 1. a) Hydrolization of Gc-protein by B and T lymphocytes' membranous enzymes into a macrophage activating factor

b) Deglycosylation of Gc protein by Nagalse from cancerous cells,leading to immunosuppression

Objectives

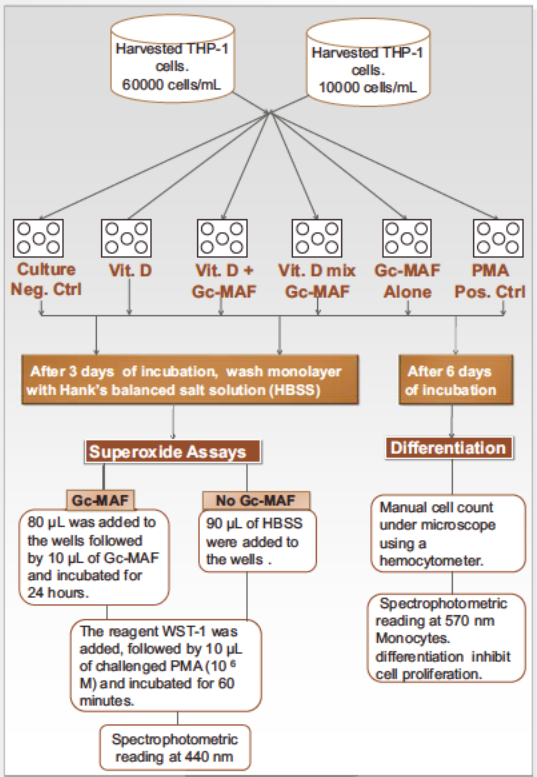
1). Maintain human monocytic leukemia cell line THP-1 in growth phase for optimal metabolic activity
2). Differentiate THP-1 cells using combinations of Vitamin D and Gc-MAF
3).Compare the superoxide anion generation of macrophages activated in the presence or absence of Gc-MAF

Materials and Methods

Culture maintenance and reagents:

RPMI-1640 culture media contained 5 % fetal bovine serum (< 0.033EU), gentamycin (antibiotic) and L-glutamine. Cells were incubated at 37° C and 5% carbon dioxide. Trypan Blue dye was used in a 1:1 ratio with harvested cells suspension, then transfered to a hemocytometer for counting under a microscope. To each well,100 µL was added to each well. Gc-MAF was obtained as a 200 ng/mL stock solution in phosphate buffered saline and 1000 pg/mL was used in the experiment. Vitamin D (cholecalciferol) stock solution (100 µM) was stored under argon and used at 0.1 µM. Gc-MAF was mixed with Vitamin D prior to cell exposure (1:1 ratio). Cell differentiation and challenging was obtained at 10 nM and 1 µM of PMA respectively.

Protocol



Results

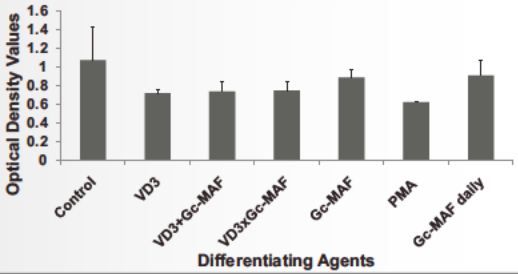


Fig. 2. Effect of vitamin D and Gc-MAF on the differentiation of THP-1 cells. The cells were treated with different combination of differentiating agents. Higher differentiation was observed with Vitamin D alone and the Vitamin D/Gc-MAF treatment, than the negative control and Gc-MAF alone. As a steroid Vitamin D up-regulates gene transcription and expression of protein kinase C-β (PKC-β) isoenzymes necessary for monocytes differentiation. Data also shows more macrophage generation with Gc-MAF alone compared to the negative control. Values are means ± S.D. of 2 experiments.

Results

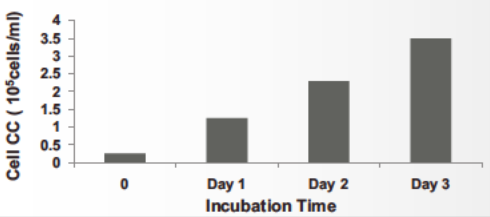


Fig. 3. Growth curve for THP-1 cell line. THP-1 cells were maintained and counted daily for three days. Data collected on day 3 shows that the cells are metabolically active due to increase in cell concentration over time.

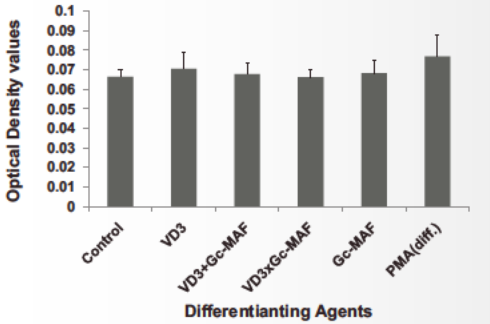


Fig. 4. Non-activated THP-1 macrophage superoxide anions production. Human monocytic leukemia cell line THP-1 were differentiated into macrophages and assayed for superoxide anions generation. Differentiation accomplished with vitamin D yielded more superoxide anions than all the other combinations and the controls. Values are means of ± S.D. for 2 experiments.

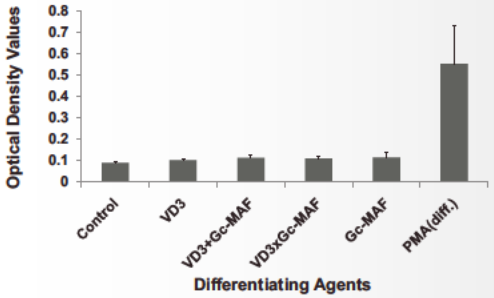


Fig. 5. Gc-MAF Activated THP-1 macrophage superoxide anions production. THP-1 macrophages were incubated for 24 hours with Gc-MAF and assayed for superoxide anions release. Differentiation accomplished with Gc-MAF releases more superoxide anions than all the other combinations including the control. Values are means of ± 2 experiments.

Results

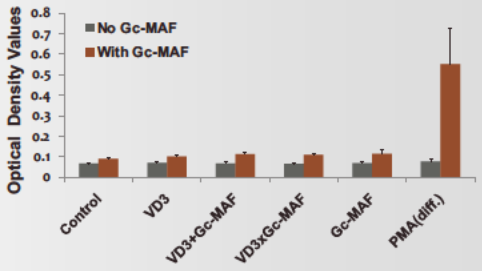


Fig. 5. Activated and non-activated THP-1 macrophage superoxide anions production. Data collected as mentioned in Materials and Methods shows that THP-1 macrophages incubated for 24 hours with Gc-MAF releases more superoxide anions than non-activated macrophages. Values are means of ± 2 experiments.

Conclusions

The experiment was designed to evaluate the effect of vitamin D and the serum glycoprotein macrophage activating factor (Gc-MAF) in differentiating monocytic leukemia cell line THP-1. Cells were maintained in growth phase for optimal metabolic activity. Superoxide oxide generation from macrophages was measured with and without Gc-MAF treatment. Vitamin D alone and combined with Gc-MAF both elicited differentiation to monocytes. Gc-MAF treatment of THP-1 monocyte stimulates cell differentiation also. Macrophages treated with Gc-MAF elicite more superoxide anions (O_2) generation than the non-treated macrophages. This suggests that patients undergoing Gc-MAF immunotherapy should have their serum vitamin D level checked and supplemented, if necessary, to maximize treatment efficiency.

Future Works

1. Isolation and purification of Vitamin D binding protein for serum Glycoprotein Macrophage Activator (Gc-MAF) synthesis
2. Human monocytic leukemia cell line THP-1 treatment with synthesized Gc-MAF
3. Cancerous cell exposure to Gc-MAF activated macrophages

References

1).Yamamoto N, Ngwenya BZ. 1987. *Activation of macrophages by lysophospholipids, and ether derivatives of neutral lipids and phospholipids. Cancer Res.*;47:2008–2013.

2).Paul, 2010. *Studies on the in Vitro Activation of Macrophages.* Florida Atlantic University Biological Department.

3).Tsuchiya et al. 1980. *Establishment and characterization of a human acute monocytic leukemia cell line* (THP-1). *Int. J. of Cancer. Volume 26, issue 2.* pages 171–176, 15 August 1980.

Acknowledgements

Dr. James Hartmann

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