Note

Effects of Bacterial Glyceroglycolipid M874B on Growth and TPA-Induced Differentiation of HL60 Cells

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Bacterial monogalactosydialcglycerol M874B (MGDAG), which protects against oxygen radicals, was found to increase the growth of the human promyelocytic leukemia cell HL60 when added to the cell culture, but suppresses the 12-O-tetradecanoyl phorbol-13-acetate-induced differentiation. Analogous MGDAG, S365B had weak, but similar effects. These activities were not observed with analogous plant glyceroglycolipids and diacylglycerol.

Key words: glyceroglycolipid; oxygen radical scavenger; HL60 cell; differentiation

In our screening for bacterial glyceroglycolipids, we found a glyceroglycolipid, M874B [1,2-di-O-(12-methyltetradecanoyl)-3-O-β-D-galactopyranosyl-sn-glycerol], isolated from Microbacterium sp. strain M874, which quenched alkyl peroxyl radicals. Further investigation showed that it protected bacterial cells from death by both exposure to heat and H$_2$O$_2$. In this respect, it has been proved that a major cause of heat killing is the superoxide radical. These results suggest that M874B is an unique oxygen radical scavenger that quenches various reactive oxygen species. Therefore, these findings encouraged us try to test it for biological effects on cell growth and differentiation of the human promyelocytic leukemia HL60. HL60 cells differentiate toward either monocyte-like cells or granulocyte-like cells associated with cessation cell growth when they are exposed to a differentiation-inducing agent. DMSO and retinoic acid induce differentiation into granulocyte lineage while 1a-25-dihydroxyvitamin D$_3$ and TPA do toward the monocytic lineage. With respect to glycolipid-related compounds, glycolipidolipids are known to modulate cell growth and transmembrane signaling during differentiation. Nojiri et al. reported that neolactoseries ganglioside and ganglioside GM3 can induce the differentiation of HL60 cells into granulocyte-like cells and macrophage-like cells, respectively. Isoda et al. showed that some kinds of microbial extracellular glycolipids also induced cell differentiation with reduced growth of HL60 cells. Recently, Ebeling et al. reported that sn-1,2-DAG, a physiological PKC activator, induced HL60 cell differentiation into the monocytic lineage, and MGDAG mimicked DAG for PKC activation. Furthermore, Yang and Shaio described how hydroxyl radical was involved in TPA-induced HL60 cell differentiation, and a hydroxyl radical scavenger, PDTC, blocked the differentiation of HL60 cells. We here tested a glyceroglycolipid M874B on TPA-induced cell differentiation and cell growth of HL60 cells and obtained the very interesting results that it could increase cell growth to a significant extent and suppress, slightly but definitely, TPA-induced cell differentiation.

The human promyelocytic leukemia HL60 cell line was obtained from Riken Cell Bank (Tsukuba, Ibaraki, Japan). The cells were regularly cultured in RPMI 1640 medium with 10% FBS in a humidified atmosphere of 5% CO$_2$ in air at 37°C. Glyceroglycolipid M874B was obtained from Microbacterium sp. strain M874 as previously described. Wheat flour MGDAG, 1,2-dimyristoyl-rac-glycerol (DAG, C14:0) and TPA were purchased from Sigma Chemicals Co. Ltd. (St. Louis, MO, U.S.A.).

For the cell growth test, HL60 cells were washed twice with serum-free RPMI 1640 medium and resuspended in a fresh RPMI 1640 medium with the indicated concentration of FBS to give a cell density of 5×10$^3$/ml. The cell suspension (100 μl) was poured into a 96-well cell culture plate and various concentrations of glycolipid solution (20 μl) or PBS were added. The glyceroglycolipid solutions in PBS were prepared by sonication and then sterilized by passing through a millipore GV 0.22 μm filter unit (Milipore Products Division, Bedford, U.S.A.). The plate was incubated in 5% CO$_2$ in air at 37°C for 6
days. Cell growth was measured by the exclusion method with trypan blue dye.

For the cell differentiation test, HL60 cells were washed twice with serum-free RPMI 1640 medium and resuspended to a cell density of 2 x 10^6/ml in the medium with 5% FBS. The fresh cell suspension of 100 µl was taken into 96-well cell culture plate and then 20 µl of 16 nM of TPA and M874B or PBS were added. The cell cultivation was done in a humidified atmosphere of 5% CO_2 in air at 37°C for 3 days. To assay the differentiation of the cells, the NBT reducing ability, which is a biochemical characteristic common for both monocytic and granulocytic differentiations, was used. Thus, 20 µl of 0.1% NBT was added to the TPA-treated HL60 cell culture, which was incubated at 37°C for 3 hr in the CO_2 incubator. The percentage of cells containing blue-black formazan deposits (NBT-reducing cells) was calculated by surveying at least 200 cells under microscope.

Figure 1 illustrates the effects of M874B and S365B on the growth of HL60 cells when they were added to the culture in RPMI 1640 medium with 10% FBS and the cell counting was measured after 3 days of cultivation. It was evident that M874B significantly increased the growth of HL60 cells, but S365B had a weak effect. The detectable effect was observed at a low M874B level of 10 µg/ml. The optimum increase in the cell growth by M874B was about 1.4-fold of the growth at the dose of approximately 100 µg/ml. On the other hand, MGDAG and DAG(C14:0) had no effect on the HL60 cell growth at levels of 50 to 250 µg/ml while M874B did increase growth (data not shown).

Figure 2 illustrates the growth profiles when HL60 cells were grown in RPMI 1640 medium with normal (10%) or a limited level (3%) of FBS in the presence or absence of 50 µg/ml M874B. The cell-growth increase caused by M874B was similar between both the cultures grown in normal and limited FBS medium.

Figure 3 illustrates the effects of M874B and S365B on HL60 cell differentiation. Less than 1% of HL60 cells were differentiated spontaneously under these conditions. About 60% of HL60 cells were induced to differentiate by treatment with TPA for 3 days under these conditions. It was evident that addition of
M874B suppressed TPA-induced cell differentiation. It decreased the differentiation rate by 40% at 100 μg/ml. The inhibition was not so large but a definite activity. S365B was a weak suppressor. Wheat MGDAG and DAG(C14:0) had no significant effects on the cell differentiation at the concentration of 50 to 250 μg/ml, at which M874B had its effect (data not shown). M874B and S365B themselves had no effect on HL60 cell differentiation in the absence of TPA.

We have demonstrated that M874B, but not wheat MGDAG, quenched a broad range of oxygen species. Therefore, it is conceivable that the effects of M874B on the growth and TPA-induced differentiation of HL60 cells is responsible for its oxygen radical scavenging activity. It could protect the cells from the surrounding reactive oxygen radicals during cultivation, so that much more growth is allowed to occur. On the other hand, the mechanism by which TPA induced a monocytic differentiation is believed to be due to activation of PKC, which also implicated in the cell regulation, differentiation, and growth. PKC inhibitors were able to block the TPA-induced differentiation. However, there is evidence that radical oxygen intermediates may act as an early important signal involved in cell activation. Yang and Shaio showed the TPA-induced monocytic differentiation of HL60 cells was specific for hydroxyl radical, not H₂O₂, because a hydroxyl radical scavenger, PDTC, blocked the differentiation. Our results with M874B seem to coincide with such an idea although PKC inhibitory activity of M874B was not examined. These findings with PDTC and M874B, however, are contrast to those with many other oxygen radical scavengers such as DMSO, flavonoids, and polyphenols, unlike PDTC and M874B, could induce cells to either monocytic or granulocytic differentiation. Detailed investigations remain discover if any such mechanism is involved in the biological functions of M874B.

References