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

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Received December 1, 1999; Accepted February 18, 2000

Bacterial monogalactosydiaoyglycerol 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-galactopyranosylsn-glycerol], isolated from Microbacterium sp. strain M874, which quenched alkyl peroxy radicals. Further investigation showed that it protected bacterial cells from death by both exposure to heat and H2O2. In this respect, it has been proved that a major cause of heat killing is the superoxide radical. These results suggest that M874B is a unique oxygen radical scavenger that quenches various reactive oxygen species. Therefore, these findings encouraged us 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 ceasing cell growth when they are exposed to a differentiation-inducing agent. DMSO and retinoic acid induce differentiation into granulocyte lineage while 1α-25-dihydroxyvitamin D3 and TPA do toward the monocytic lineage. With respect to glycolipid-related compounds, glycosphingolipids 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 monocyctic lineage, and MGDAG mimicked DAG for PKC activation. Furthermore, Yang and Shaik 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% CO2 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 millex GV 0.22 μm filter unit (Milipore Products Division, Bedford, U.S.A.). The plate was incubated in 5% CO2 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 $2 \times 10^6$/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% CO₂ 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.12 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 CO₂ 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

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Fig. 1. Effects of M874B and S365B on Growth of HL60 Cells. HL60 cells were cultivated at 37°C for 3 days in RPMI 1640 medium with 10% FBS in the presence of various concentrations of M874B or S365B. Cells were counted by the exclusion method with trypan blue dye. Each value is expressed as the mean ± SD of triplicate experiments. (■), M874B; (▲), S365B.

Fig. 2. Growth Increasing Effect of M874B on HL60 Cells in Limited (3%) or Non-limited (10%) FBS Medium. HL60 cells were cultivated in RPMI 1640 medium with 10% or 3% FBS in the presence of 50 µg/ml of M874B. Cells were counted by the exclusion method with trypan blue dye. Values are means of triplicate assays. Standard deviations were <10%. 10% FBS medium: (○), none; (●), M874B (50 µg/ml); 3% FBS medium: (□), none; (■), M874B (50 µg/ml).

Fig. 3. Effects of M874B and S365B on TPA-Induced HL60 Cell Differentiation. HL60 cells were cultivated in RPMI 1640 medium with 5% FBS in the presence of about 2 nM TPA and M874B or S365B at 37°C for 3 days. Differentiation is expressed as a percentage of NBT-reduced cells by microscopic survey of 200 cells. Fisher's test was done to detect the significant differences among all treatments. Each value is expressed as the mean ± SD of triplicate experiments. (■), M874B; (▲), S365B. *: p<0.05.
M874B suppressed TPA-induced cell differentiation. It decreased the differentiation rate by 40% at 100 µg/ml. The inhibition was not 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 that 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 though PKC inhibitory activity of M874B was not examined. These findings with PDTC and M874B, however, are contrast to those with many other oxygen reactive 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