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# Suppressive Effects of Swainsonine and *N*-Butyldeoxynojirimycin on Human Bone Marrow Neutrophil Maturation

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**The effects of the N-linked oligosaccharide inhibitors swainsonine and *N*-butyldeoxynojirimycin (NB-DNJ) on granulopoiesis was investigated using human bone marrow cells in *in vitro* liquid and agar cultures. The addition of the inhibitors into cultures containing granulocyte colony-stimulating factor (G-CSF) suppressed maturation from myelocytes into mature neutrophils. Swainsonine did not induce apoptosis, but NB-DNJ induced considerable apoptosis, especially in the presence of G-CSF. This result indicated that the decrease of mature neutrophils by swainsonine was not because of cell degeneration. In the case of NB-DNJ, it was thought to be because of both maturation suppression and apoptosis. In a colony-forming unit-granuloid (CFU-G) colony assay, the number of colonies was increased in the presence of the inhibitors, but the morphology of colonies was predominantly compact, or immature. The inhibitors also suppressed the expressions of mRNAs of CCAAT/enhancer binding protein ϵ (C/EBPϵ) and G-CSF receptor as markers of terminal neutrophil maturation. These findings suggested that the incompleteness of N-linked oligosaccharide leads to the suppression of terminal neutrophil maturation.** 

Most secretory and membrane proteins in mammalian cells have the complex type of N-linked oligosaccharide attached to appropriate asparagine residue. The complex type of N-linked oligosaccharide is biosynthesized via high-mannose and hybrid intermediates by the action of enzymes specific to each process (1). Swainsonine inhibits α-mannosidase II and leads to a hybrid type of glycoproteins (2–5). *N*-Butyldeoxynojirimycin (NB-DNJ) prevents the removal of glucose residue by the inhibition of glucosidase I and glucosidase II and leads to a high-mannose type of glycoproteins (6, 7). It has been demonstrated that swainsonine exhibits various biological effects such as anti-cancer activity (8–11), the enhancement of macrophage tumoricidal activity (12), natural killer activity (13), the enhancement of IL-2-mediated lymphocyte mitogenesis (14), and the protection of bone marrow cells during cytotoxic chemotherapy (15, 16). On the other hand, the analysis of patients with HEMPAS (hereditary erythroblastic multinuclearity with positive acidified serum lysis) has provided the interesting findings that the membrane proteins in erythroid cells have a hybrid type of polylactosaminoglycans and that the cause of incomplete glycosylation is a defect of Golgi α-mannosidase II activity, subsequently leading to ineffective erythropoiesis (17). The knockout mice of α-mannosidase II also exhibited ineffective erythropoiesis, similar to what was noted in HEMPAS patients (19). These findings suggest that the hybrid type of oligosaccharide leads to the disturbance of maturation in erythroid cells.

NB-DNJ has been considered an experimental or clinical drug against human immunodeficiency virus-1 (20–22) and hepatitis B virus (23) because normal N-glycosylation of the surface proteins of these viruses is essential for the formation of an infectious virus. NB-DNJ also inhibits the ceramide-specific glucosyltransferase which catalyzes the first step in glycosphingolipid biosynthesis (7). Therefore, NB-DNJ has also been suggested as an agent for the treatment of type 1 Gaucher disease (24), Sandhoff disease (25), and Tay-Sachs disease (26), which are the glycosphingolipid lysosomal storage diseases. There are, however,

Abbreviations used: NB-DNJ, *N*-butyldeoxynojirimycin; G-CSF, granulocyte colony-stimulating factor; G-CSFR, G-CSF receptor; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; C/EBPϵ, CCAAT/enhancer binding protein ϵ; PI, propidium iodine; CFU-G, colony-forming unit-granuloid.

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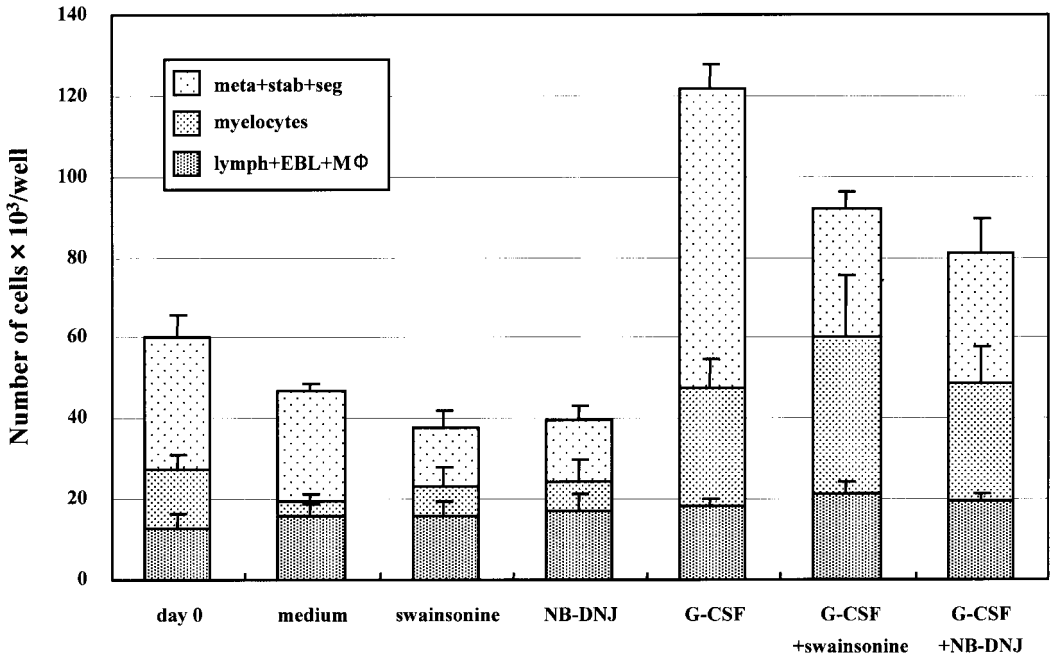


**FIG. 1.** After nonadherent bone marrow cells ($6 \times 10^4$/well) were cultured in triplicate with swainsonine (1 $\mu$g/ml) or NB-DNJ (15 $\mu$g/ml) in the presence or absence of G-CSF (100 ng/ml) for 7 days, the cell numbers were counted and the differentials were scored with Wright–Giemsa stain. Abbreviations are as follows: Meta, metamyelocytes; stab, stab cells; seg, segmented cells; lymph, lymphocytes; EBL, erythroblasts, M$\phi$, macrophages. Each bar represents the mean ± standard errors of data from three different experiments, respectively.

very few reports concerning the effects of NB-DNJ on hematopoiesis.

Considering that these N-linked oligosaccharide inhibitors could be clinically applied as therapeutic agents, it is important to investigate the effects of the inhibitors on hematopoiesis. In this study, we have focused on the effects of N-glycosylation inhibitors on granulopoiesis, especially on the process of neutrophil maturation. We have studied the effects of the inhibitors on human bone marrow cells by estimating the changes in the number of cells and granuloid colonies and the changes in the expression of mRNAs of CCAAT/enhancer binding protein epsilon (CEBP/$\epsilon$) (27–30) and granulocyte colony-stimulating factor (G-CSF) receptor (31, 32) as markers of terminal neutrophil maturation. We present the first published evidence that the treatment of human bone marrow by swainsonine or NB-DNJ *in vitro* leads to the suppression of neutrophil maturation.

## MATERIALS AND METHODS

*Reagents.* Swainsonine, NB-DNJ, and tunicamycin from Wako Pure Chemical Industries Ltd., Osaka, Japan, were dissolved in 100% dimethyl sulfoxide (DMSO; Nakarai Chemicals Ltd., Kyoto, Japan) and diluted to an appropriate concentration with Iscove's modified Dulbecco's medium (IMDM; Gibco Laboratories, Grand Island, NY). The final concentration of DMSO in cultures was set below 0.1%. Recombinant human granulocyte colony stimulating factor (G-CSF) was kindly provided by Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). Fluorescein isothiocyanate (FITC)-conjugated recombinant human annexin V and propidium iodine were purchased from Caltag Laboratories (Burlingame, CA) and Wako Pure Chemical Industries Ltd., respectively.

*Nonadherent bone marrow cell preparation and cell culture.* Human bone marrow blood was obtained, with informed consent, from patients with malignant lymphoma who had no invasion in the bone marrow. Bone marrow mononuclear cells were separated by density gradient centrifugation on lymphocyte separation medium (LSM; density 1.077: Litton Bionetics Inc., Charleston, SC). Most of the segmented neutrophils were removed by this procedure. After the cells were incubated in IMDM containing 20% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS) for 2 h at 37°C in plastic dishes to remove monocytes, nonadherent bone marrow mononuclear cells were collected from supernatants and used as target cells for further study. Nonadherent bone marrow cells ($6 \times 10^4$ cells/well) were cultured with 250 $\mu$l of IMDM containing 30% FBS and 10 mg/ml deionized bovine serum albumin (BSA; Cohn fraction V, Sigma Chemical Co., St. Louis, MO) in 96-well round-bottomed microplates (Nunc, Denmark, Holland). In this liquid system, swainsonine, NB-DNJ, or tunicamycin was added at a final concentration of 1 $\mu$g/ml, 15 $\mu$g/ml, or 1 $\mu$g/ml, respectively, in the presence or absence of 100 ng/ml of G-CSF. After 7 days incubation at 37°C in humidified 5% $CO_2$ in air, the number of vital cells in each well was counted by the trypan blue exclusion method. The parts of the cultured cells were stained with Wright–Giemsa and used for flow cytometric analysis of apoptosis using annexin V and propidium iodine. In the parts experiment, the cells were collected at day 0, day 2, and day 4 of incubation and stored at −80°C for total RNA extraction.

*Colony-forming unit-granuloid (CFU-G) colony assay.* Nonadherent bone marrow cells ($2 \times 10^5$ cells/plate) were cultured in 1 ml vol of culture medium in 35 × 10 mm plastic petri dishes (Falcon Plastics, Oxnard, CA) at 37°C in humidified 5% $CO_2$ in air. The culture medium consisted of 0.38% agar (Difco Laboratories, Detroit,

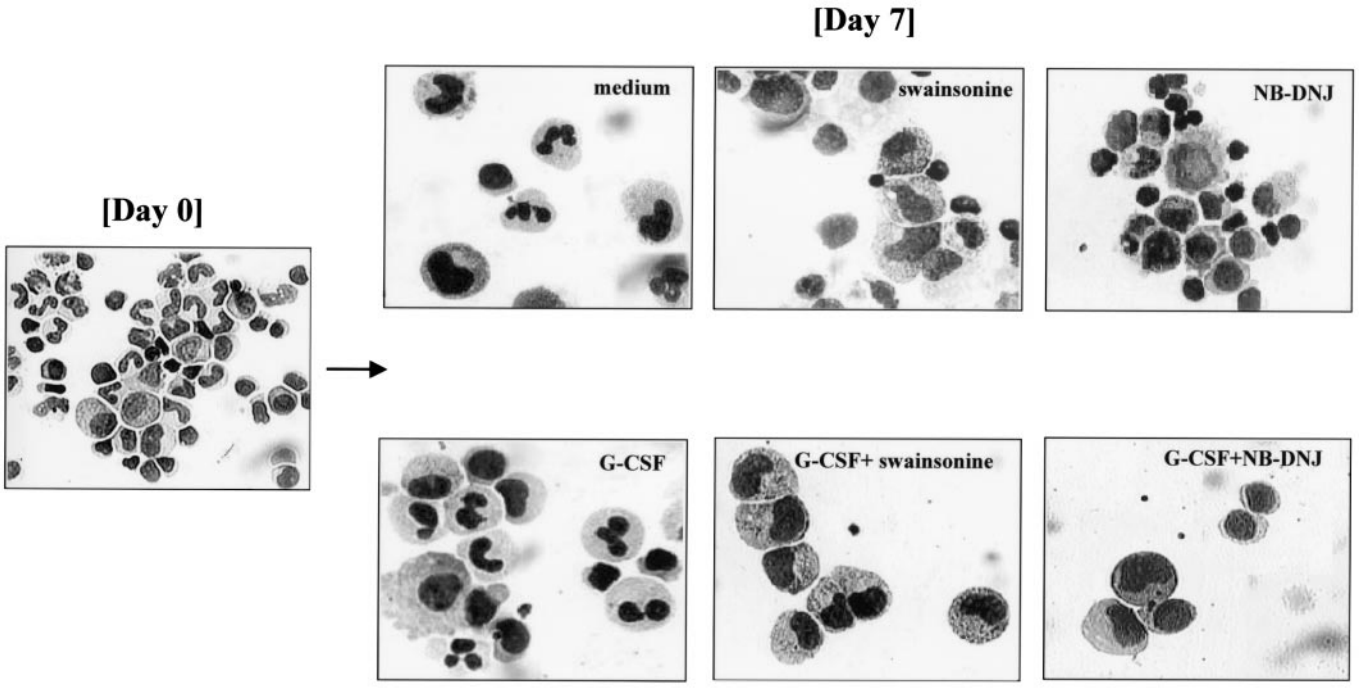


**FIG. 2.** These photos depict the represented cells on day 0 and after 7 days of culturing under the same conditions as in Fig. 1. Segmented cells were not found before culturing began (day 0). In the culture containing medium alone, a few segmented cells appeared (medium on day 7). G-CSF stimulated neutrophil maturation and increased the number of segmented cells (G-CSF on day 7). In the culture containing swainsonine or NB-DNJ (G-CSF + swainsonine on day 7 or G-CSF + NB-DNJ on day 7), myelocytes are predominantly observed and matured neutrophils appeared in lower numbers than in the cultures containing G-CSF alone. Magnifications are 600× in the photo of day 0 and 1000× in the photos of day 7.

MI), 30% FBS, 10 mg/ml BSA, 100 ng/ml G-CSF, and IMDM. In this agar culture system, swainsonine or NB-DNJ was added at a final concentration of 1 $\mu$g/ml or 15 $\mu$g/ml, respectively. After 11 days of incubation, the number or morphology of colonies was observed under an inverted microscope. To identify the cells that formed the colonies, the cells were picked out from each colony, smeared on the slide glasses, and examined with Wright–Giemsa staining and esterase double staining using naphthol AS-D chloroacetate and $\alpha$-naphthyl butyrate as substrates (Muto Chemical Co. Ltd., Tokyo, Japan).

*Flow cytometric analysis of early and late apoptosis.* Two hundred fifty microliters of culture medium containing the cultured cells ($10^4$ to $10^5$) was mixed with 250 $\mu$l of PBS, 20 $\mu$l of 50 mM $CaCl_2$, 5 $\mu$l of 200 $\mu$g/ml FITC-conjugated annexin V, and 5 $\mu$l of 200 $\mu$g/ml propidium iodine. After 15-min incubation at room temperature in the dark, flow cytometric analysis was carried out. The cells that have bound annexin V only are early apoptotic cells. The cells that take up propidium iodine are late apoptotic or necrotic cells. The population of cells that are negative for both annexin V and propidium iodine are normal vital cells (33).

*RT-PCR for C/EBP$\epsilon$, G-CSF receptor, and G3PDH.* Total RNA was extracted using RNAzol (Wako Pure Chemical Industries Ltd.) according to the manufacturer's instructions. In some experiments, segmented neutrophils were collected from a healthy donor and used for total RNA extraction in addition to cultured cells. Reverse transcriptase-polymerase chain reaction (RT-PCR) was done in 50 $\mu$l of total volume using a kit (Access of RT-PCR) of Promega Corporation, Madison, WI. This system uses avian myeloblastosis virus (AMV) reverse transcriptase for first strand cDNA synthesis and thermostable *Tfl* DNA polymerase from *Thermus flavus* for second strand cDNA synthesis and DNA amplification. Ten nanograms of total RNA was used as templates for RT-PCR after denaturation at 94° C for 2 min. Each downstream primer specific to each gene was used for first strand cDNA synthesis. RT-PCR to detect the expression of each mRNA of C/EBP$\epsilon$. G-CSF receptor, and G3PDH (glyceraldehyde-3-phosphate dehydrogenase) was performed with the following upstream and downstream oligonucleotide primers: 5′-TGCAGTACCAAGTGGCACACT-3′ (nt 416–436, nt; the number from the translation-initiation site of each gene) and 5′-ATGTACTCCAGCACCTTCTGC-3′ (nt 1379–1359) for C/EBP$\epsilon$ (27), 5′-ACCTGGGCACAGCTGGAGTGG-3′ (nt 1621–1641) and 5′- CAGGCTGCTGTGAGCTGGGTCTGG-3′ (nt 2010–1987) for G-CSF receptor (31), and 5′-CCCATGTTCGTCATGGGTGTGAAC-3′ (nt 379–402) and 5′-GAGCTTCCCGTTCAGCTCAGGGAT-3′ (nt 678–655) for G3PDH (34). The RT-PCR was performed under the following conditions: 48° C, 45 min for synthesis of first strand cDNA, 94° C, 2 min for AMV reverse transcriptase inactivation and RNA/cDNA/primer denaturation, 40 cycles of 94° C, 30 sec, 60° C, 1 min, and 68° C, 2 min for second strand cDNA synthesis and PCR amplification, and 68° C, 7 min for final extension. To control for sample-to-sample variation in the quantity of mRNA and variation in the RT and PCRs, the mRNA levels of G3PDH, a housekeeping gene, were determined in parallel.

## RESULTS

### *Effects of Swainsonine and NB-DNJ on the Proliferation and Maturation of Nonadherent Bone Marrow Cells in the Presence or Absence of G-CSF*

The changes in the cell number and the differentials before and after culturing are depicted in Figs. 1 and 2. In Fig. 1, neutrophils are divided into two categories,

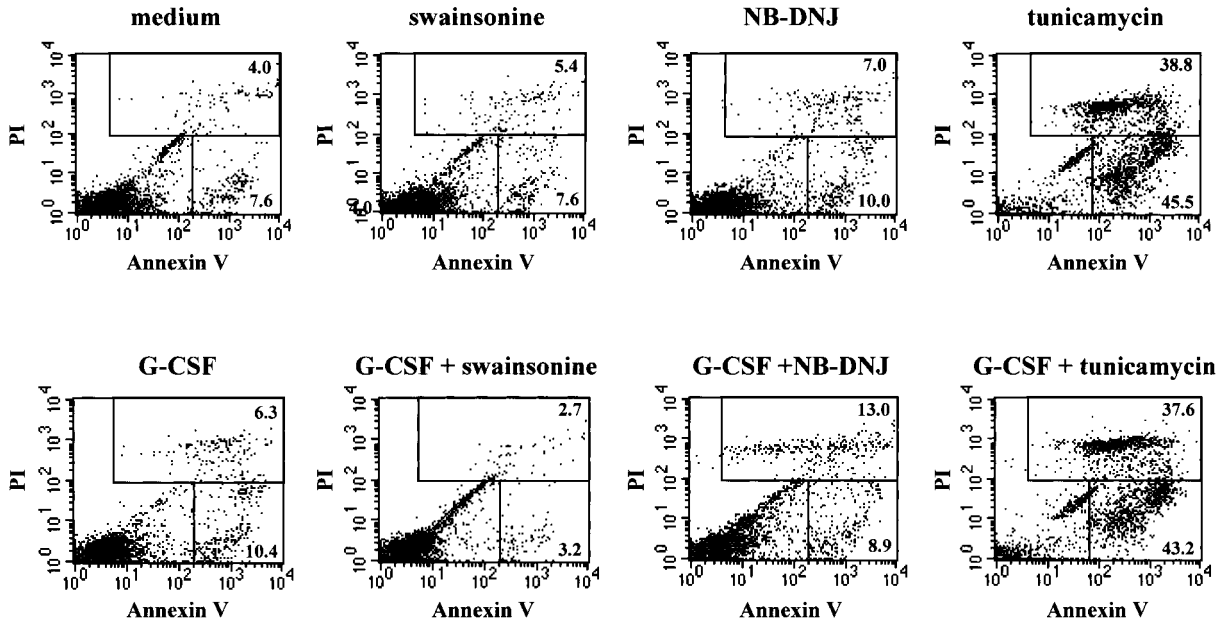


**FIG. 3.** Nonadherent bone marrow cells were cultured with swainsonine, NB-DNJ, and tunicamycin in the presence or absence of G-CSF under the same conditions as shown in Fig. 1 for 7 days and then stained with FITC-conjugated annexin V and propidium iodine (PI) and analyzed by flow cytometry. The numbers (%) in the lower and upper boxes show early and late apoptotic cells, respectively.

the proliferative compartment, including predominantly myelocytes, and the maturation compartment, including metamyelocytes, stab cells, and segmented cells. There were almost no segmented neutrophils in the nonadherent bone marrow cells before culturing (on day 0). On day 7 of culturing, spontaneous maturation into segmented cells was observed in cultures containing medium alone. G-CSF stimulated both the neutrophil maturation and the proliferation of myelocytes. The presence of swainsonine and NB-DNJ leads to the decrease in the number of cells in the maturation compartment but not in the proliferative compartment. This finding indicated that swainsonine and NB-DNJ suppressed the differentiation and maturation of myelocytes into mature neutrophils and led to a decrease in the total cell number. The total number of other blood cells, including lymphocytes, erythroblasts, and macrophages, did not change significantly.

### *Effects of Swainsonine, NB-DNJ, and Tunicamycin on Early and Late Apoptosis of Bone Marrow Cells in the Presence or Absence of G-CSF*

To examine whether the decrease in the cell number by swainsonine or NB-DNJ is a result of the increase of apoptotic cells, the cultured cells were analyzed by flow cytometry using FITC-conjugated annexin V and propidium iodine. The effects of tunicamycin were also examined. As shown in Fig. 3, the percentage of early and late apoptotic cells were 7.6% and 4.0% for medium alone, 7.6% and 5.4% for swainsonine, 10.0% and 7.0% for NB-DNJ, 45.5% and 38.8% for tunicamycin, 10.4% and 6.3% for G-CSF, 3.2% and 2.7% for G-CSF plus swainsonine, 8.9% and 13.0% for G-CSF plus NB-DNJ, and 43.2% and 37.6% for G-CSF plus tunicamycin, respectively. These data indicated that the decrease of mature neutrophils by swainsonine was not due to the increase in the number of apoptotic cells, and, in the case of DB-DNJ, the decrease was in part due to apoptosis. In the culture containing tunicamycin, many cells were damaged and the surviving cells were confirmed to be lymphocytes only on smear.

### *Effects of Swainsonine and DB-DNJ on Granuloid Colony Formation*

The effects of swainsonine and DB-DNJ on the proliferation and differentiation of granuloid progenitor cells were examined using agar culture assay. Any colonies formed until day 7 in any of the cultures were still of a compact morphology (data not shown). This type of colony indicates that the process of differentiation and maturation of progenitors do not reach the segmented neutrophils, which can migrate around the colonies. On day 11, almost all colonies stimulated with G-CSF became diffuse colonies (see the lower example in Fig. 4A). The presence of swainsonine or DB-DNJ increased significantly the number of colonies compared to the number in medium alone (see Fig. 4B), but the colonies were predominantly compact (see the upper example in Fig. 4A). It was ascertained with Wright–Giemsa staining and esterase double staining that the cells forming diffuse or compact colonies were neutrophils in both cases (data not shown).

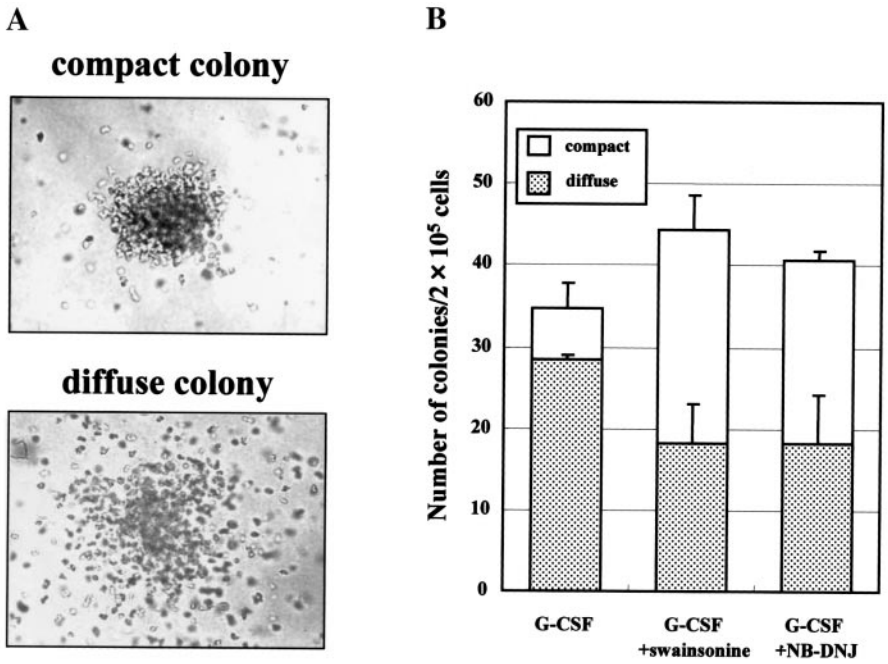


**FIG. 4.** The left photo (A) shows two types of colonies formed, referred to as the diffuse colony (lower) and the compact colony (upper). Diffuse colonies were predominantly observed in the culture containing G-CSF alone. Compact colonies occurred in greater frequency in the culture containing G-CSF plus swainsonine or NB-DNJ. The figure on the right (B) shows the number of colonies formed. Each bar represents the mean ± standard errors of the number of colonies/2 × $10^5$ cells from triplicate cultures.

*Expression of mRNAs of C/EBPε and G-CSF Receptor as Markers of Mature Neutrophils*

First, we checked the differences of mRNA expression of C/EBPε and G-CSF receptor between nonadherent bone marrow cells and peripheral segmented neutrophils. As shown in Fig. 5A, those mRNAs were highly expressed in segmented neutrophils compared to bone marrow cells. It was shown that the expression of those mRNAs could be markers of terminal maturation of neutrophils. Figure 5B shows the mRNA expression in nonadherent bone marrow cells cultured with medium alone, G-CSF, G-CSF plus swainsonine, and G-CSF plus NB-DNJ for 2 and 4 days. G-CSF stimu-

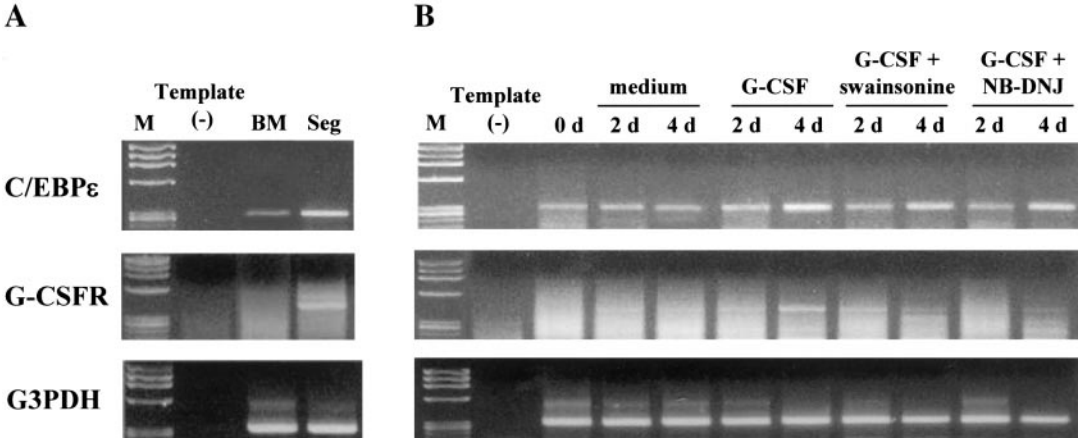


**FIG. 5.** The figure on the left (A) shows the expression of mRNAs of C/EBPε, G-CSF receptor, and G3PDH in the nonadherent bone marrow cells and peripheral segmented neutrophils. The figure on the right (B) shows the expression of the same mRNAs expressions at day 0, day 2, and day 4. The nonadherent bone marrow cells were cultured with medium alone, G-CSF (100 ng/ml), G-CSF (100 ng/ml) plus swainsonine (1 μg/ml), and G-CSF (100 ng/ml) plus NB-DNJ (15 μg/ml) for 2 days and 4 days. Ten microliters of each amplification reaction by RT-PCR was separated on a 2% agarose gel in 1× TBE buffer containing 0.5 μg/ml ethidium bromide. The specific product is 308 bp for C/EBPε, 390 bp for G-CSF receptor, and 300 bp for G3PDH. Abbreviations are as follows: G-CSFR, G-CSF receptor; M, φX174 *Hae*III marker.

lated the expression of those mRNAs, but the expressions were suppressed in the presence of swainsonine or NB-DNJ.

## DISCUSSION

The modifications of N-linked oligosaccharide structure by swainsonine or NB-DNJ lead to changes in cell–cell and cell–matrix interactions and influence various biological events. With regard to erythropoiesis, the defective maturation has been observed in patients with HEMPAS (17, 18) or knockout mice with defective $\alpha$-mannosidase II activity (19). In the present study, we have focused on the effects of swainsonine or NB-DNJ on human bone marrow neutrophils in liquid and agar cultures *in vitro*. It has been well known that marrow neutrophils are divided into the mitotic, or proliferative, compartment and the maturation compartment. Myeloblasts, promyelocytes, and myelocytes are capable of replication and constitute the mitotic compartment. Metamyelocytes, stab cells, and segmented neutrophils, none of which replicate, constitute the maturation storage compartment (35). The present study has shown that the maturation compartment was decreased in the presence of swainsonine or NB-DNJ. The flow cytometric analysis showed that the number of apoptotic cells did not increase in the presence of swainsonine compared to medium alone or G-CSF alone, whereas NB-DNJ induced apoptosis, especially when added together with G-CSF in cultures. Therefore, the present results suggested that the decrease of mature neutrophils caused by swainsonine or NB-DNJ was the result of the suppression of proliferation and differentiation from myelocytes into metamyelocytes but not the result of cell degeneration by apoptosis. In the case of NB-DNJ, the decrease was thought to be caused by both maturation suppression and apoptosis.

Recent studies have also shown that swainsonine and NB-DNJ are not cytotoxic to cultured cells, but tunicamycin, an inhibitor of the first step in the biosynthesis of *N*-linked oligosaccharide, induces cell death in various cultured cells (36–39). We also recognized that tunicamycin at a final concentration of 1 $\mu$g/ml induced marked apoptosis in bone marrow cells and made no granuloid colonies (data not shown). Elbein *et al.* reported that swainsonine at levels of up to 1 $\mu$g/ml did not affect the growth rate, cell size, or cell shape in cell lines, including Madin-Darby canine kidney cells, Chinese hamster ovary cells, simian virus-181 cells, B-16 melanoma cells, and intestine 407 cells over a 5-day period (40). In the clinical use of swainsonine (50–550 $\mu$g/kg/day) in patients with advanced malignancies, neither lympho- nor myelo-suppression were observed over the 5-day infusion (9, 10). The discrepancy between those clinical data and our data concerning the effect of swainsonine on granulopoiesis *in vitro* seems to exist because the feedback mechanisms for neutrophils homeostasis are functioning *in vivo*.

Regarding NB-DNJ, Neises *et al.* have demonstrated that treatment of HL-60 cells with NB-DNJ results in several morphological changes within the cell, especially in the Golgi apparatus, but all the observed changes are fully reversible on withdrawal of the compound (41). Bieberich *et al.* have also reported that apoptosis of neuroblastoma NG108-15 cells is not observed on incubation with NB-DNJ, and its use is suggested to be less toxic for treatment of Gaucher's disease and other sphingolipid storage disorders (24). However, in a phase I study of NB-DNJ (8–64 mg/kg/day) in patients with advanced HIV disease, Grade III leukopenia and neutropenia were seen in 7 out of 29 patients (20). These clinical findings and our *in vitro* data indicated that NB-DNJ may induce apoptosis on neutrophilic cells under certain conditions and that during clinical use of NB-DNJ attention should be paid to neutropenia when G-CSF is used together with it.

Previous reports have shown that swainsonine leads to an increase in myeloid colony forming cells *in vivo* and *in vitro* and is useful for the protection of hematopoietic systems from chemotherapeutic toxicity (11, 15, 16, 42). In colony assays, the addition of swainsonine enhanced the granuloid colony formation compared to the use of G-CSF alone, in accordance with those earlier reports. NB-DNJ also increased the colony number. To date, there have been no reports on the effect of NB-DNJ on granuloid progenitor cells. We found that the type of colonies formed in the presence of swainsonine or NB-DNJ was predominantly compact colonies, which meant that maturation from myelocytes into segmented neutrophils was suppressed and that the colonies were formed from immature neutrophils. These results suggested that the modification of *N*-oligosaccharide by swainsonine or NB-DNJ stimulated the proliferation of granuloid progenitors but suppressed the terminal maturation.

To further test the suppression of maturation by swainsonine or NB-DNJ, we examined the expression of C/EBP$\epsilon$ mRNA and G-CSF receptor mRNA as terminal neutrophil differentiation markers. Yamanaka *et al.* have demonstrated that human normal hematopoietic stem cells (CD34+) do not express significant levels of C/EBP$\epsilon$, but the expression is detected in granulocytic cells after 8 days in culture containing G-CSF (29). Tkatch *et al.* have also shown that G-CSF receptor mRNA levels are maintained by G-CSF in peripheral neutrophils but not in bone marrow cells, and that the differentiation of acute promyelocytic cell line NB4 or myeloid leukemia cell line HL-60 by all-trans retinoic acid results in a striking increase in G-CSF receptor mRNA expression (32). It was also demonstrated in this study that purified segmented cells expressed much higher levels of C/EBP$\epsilon$ mRNA

and G-CSF receptor mRNA than did nonadherent bone marrow cells, including immature neutrophils, lymphocytes, and erythroblasts. When nonadherent bone marrow cells were cultured with G-CSF, the expression of those mRNAs was strongly enhanced. The addition of swainsonine suppressed that expression as well as suppressing the production of segmented neutrophils.

The present results suggest that the modification of N-linked oligosaccharide structure by swainsonine or NB-DNJ suppresses the terminal maturation of neutrophils, especially at the stage when myelocytes are becoming metamyelocytes. This work is thought to be a first step in elucidating the critical factors in neutrophil maturation and the causes of chronic neutropenia with maturation arrest or myelodysplastic syndrome with ineffective hematopoiesis.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Moremen, K. W., Trimble, R. B., and Herscovics, A. (1994) *Glycobiology* **4,** 113–125.
2. Tulsiani, D. R. P., Harris, T. M., and Touster, O. (1982) *J. Biol. Chem.* **257,** 7936–7939.
3. Tulsiani, D. R., and Touster, O. (1983) *J. Biol. Chem.* **258,** 7578–7585.
4. Elbein, A. D. (1991) *FASEB J.* **5,** 3055–3063.
5. Kang, M. S., Bowlin, T. L., Vijay, I. K., and Sunkara, S. P. (1993) *Carbohydr. Res.* **248,** 327–337.
6. Saunier, B., Kilker, R. D. Jr., Tkacz, J. S., Quaroni, A., and Herscovics, A. (1982) *J. Biol. Chem.* **257,** 14155–14161.
7. Platt, F. M., Neises, G. R., Dwek, R. A., and Butters, T. D. (1994) *J. Biol. Chem.* **269,** 8362–8365.
8. Dennis, J. W. (1991) *Semin. Cancer Biol.* **2,** 411–420.
9. Goss, P. E., Baptiste, J., Fernandes, B., Baker, M., and Dennis, J. W. (1994) *Cancer Res.* **54,** 1450–1471.
10. Goss, P. E., Reid, C. L., Bailey, D., and Dennis, J. W. (1997) *Clin. Cancer Res.* **3,** 1077–1086.
11. Roberts, J. D., Klein, J. L., Palmantier, R., Dhume, S. T., George, M. D., and Olden, K. (1998) *Cancer Detect. Prev.* **22,** 455–462.
12. Das, P. C., Roberts, J. D., White, S. L., and Olden, K. (1995) *Oncol. Res.* **7,** 425–433.
13. Yagita, M., Noda, I., Maehara, M., Fujieda, S., Inoue, Y., Hoshino, T., and Saksela, E. (1992) *Int. J. Cancer* **52,** 664–672.
14. Bowlin, T. L., and Sunkara, P. S. (1988) *Biochem. Biophys. Res. Commun.* **l51,** 859–864.
15. Oredipe, O. A., White, S. L., Grzegorzewski, K., Gause, B. L., Cha, J. K., Miles, V. A., and Olden, K. (1991) *J. Natl. Cancer Inst.* **83,** 1149–1156.
16. Klein, J. L., Roberts, J. D., George, M. D., Kurtzberg, J., Breton, P., Chermann, J. C., and Olden, K. (1999) *Br. J. Cancer* **80,** 87–95.
17. Fukuda, M. N., Masri, K. A., Dell, A., Luzatto, L., and Moremen, K. W. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7443–7447.
18. Fukuda, M. N. (1993) *Baillieres Clin. Haematol.* **6,** 493–511.
19. Chui, D., Oh-Eda, M., Liao, Y. F., Panneerselvam, K., Lal, A., Marek, K. W., Freeze, H. H., Moremen, K. W., Fukuda, M. N., and Marth, J. D. (1997) *Cell* **90,** 157–167.
20. Tierney, M., Pottage, J., Kessler, H., Fischl, M., Richman, D., Merigan, T., Powderly, W., Smith, S., Karim, A., Sherman, J., *et al.* (1995) *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **10,** 549–553.
21. Fischer, P. B., Karlsson, G. B., Dwek, R. A., and Platt, F. M. (1996) *J. Virol.* **70,** 7153–7160.
22. Fenouillet, E., Papandreou, M. J., and Jones, I. M. (1997) *Virology* **231,** 89–95.
23. Mehta, A., Lu, X., Block, T. M., Blumberg, B. S., and Dwek, R. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 1822–1827.
24. Bieberich, E., Freischutz, B., Suzuki, M., and Yu, R. K. (1999) *J. Neurochem.* **72,** 1040–1049.
25. Jeyakumar, M., Butters, T. D., Cortina, Borja, M., Hunnam, V., Proia, R. L., Perry, V. H., Dwek, R. A., and Platt, F. M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 6388–6393.
26. Platt, F. M., Neises, G. R., Reinkensmeier, G., Townsend, M. J., Perry, V. H., Proia, R. L., Winchester, B., Dwek, R. A., and Butters, T. D. (1997) *Science* **276,** 428–431.
27. Chumakov, A. M., Grillier, I., Chumakova, E., Chih, D., Slater, J., and Koeffler, H. P. (1997) *Mol. Cell. Biol.* **17,** 1375–1386.
28. Yamanaka, R., Barlow, C., Lekstrom, H. J., Castilla, L. H., Liu, P. P., Eckhaus, M., Decker, T., Wynshaw, B. A., and Xanthopoulos, K. G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13187–13192.
29. Yamanaka, R., Kim, G. D., Radomska, H. S., Lekstrom, H. J., Smith, L. T., Antonson, P., Tenen, D. G., and Xanthopoulos, K. G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6462–6467.
30. Morosetti, R., Park, D. J., Chumakov, A. M., Grillier, I., Shiohara, M., Gombart, A. F., Nakamaki, T., Weinberg, K., and Koeffler, H. P. (1997) *Blood* **90,** 2591–2600.
31. Fukunaga, R., Seto, Y., Mizushima, S., and Nagata, S. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8702–8706.
32. Tkatch, L. S., Rubin, K. A., Ziegler, S. F., and Tweardy, D. J. (1995) *J. Leukocyte Biol.* **57,** 964–971.
33. Koopman, G., Reutelingsperger, C. P., Kuijten, G. A., Keehnen, R. M., Pals, S. T., and van Oers, M. H. (1994) *Blood* **84,** 1415–1420.
34. Tso, J. Y., Sun, X. H., Kao, T. H., Reece, K. S., and Wu, R. (1985) *Nucleic Acids Res.* **13,** 2485–2502.
35. Bernard, M. B., and Golde, D. W. (1995) Production, distribution, and fate of neutrophils. *in* Williams Hematology (Beutler, E., Lichtman, M. A., Coller, M. S., and Kipps, T. J., Eds.), 5th ed., pp. 773–779, McGraw–Hill, New York.
36. Perez, S. D., and Mollinedo, F. (1995) *J. Cell. Physiol.* **163,** 523–531.
37. Dricu, A., Carlberg, M., Wang, M., and Larsson, O. (1997) *Cancer Res.* **57,** 543–548.
38. Walker, B. K., Lei, H., and Krag, S. S. (1998) *Biochem. Biophys. Res. Commun.* **250,** 264–270.
39. Martinez, J. A., Torres, N. I., Amigo, L. A., and Banerjee, D. K. (1999) *Cell. Mol. Biol. Noisy-le-grand.* **45,** 137–152.
40. Elbein, A. D., Pan, Y. T., Solf, R., and Vosbeck, K. (1983) *J. Cell. Physiol.* **115:**265–275.
41. Neises, G. R., Woodman, P. G., Butters, T. D., Ornberg, R. L., and Platt, F. M. (1997) *Biol. Cell.* **89,** 123–131.
42. White, S. L., Nagai, T., Akiyama, S. K., Reeves, E. J., Grzegorzewski, K., and Olden, K. (1991) *Cancer Commun.* **3,** 83–91.