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Interleukin-3-associated ganglioside GD1a is induced independently of normal interleukin-3 receptor in murine myelogenous leukaemia NFS60 cells transfected with the interleukin-3 gene

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The mechanism of interleukin-3 (IL-3) independent cell growth and of IL-3-associated ganglioside expression was analysed using the IL-3 dependent murine myelogenous leukaemia cell line NFS60-I7 and *IL-3* gene-transfected sublines. The transfected cell lines showed autonomous cell growth, tumorigenicity, and IL-3 associated ganglioside GD1a expression in spite of their IL-3 production. While the parental NFS60-I7 cells did not express significant amounts of GD1a, exogenous recombinant IL-3 (rIL-3) was demonstrated to induce IL-3-associated ganglioside GD1a expression in NFS60-I7 cells. Furthermore, the growth potential of the transfected cells was not blocked by anti-IL-3 antibody and expression of GD1a was not affected by anti-IL-3 antibody. These findings suggest that IL-3 expressed intracellularly by gene transfection might act independently of the normal IL-3 receptor on autonomous cell growth and on IL-3-associated GD1a expression in murine myelogenous leukaemia NFS60 cells.

Keywords: interleukin-3, ganglioside GD1a, factor dependent cell line, gene transfection, autocrine mechanism

Abbreviations: IL-3, interleukin-3; rIL-3, recombinant interleukin-3; FCS, fetal calf serum; PWM-SCCM, pokeweed mitogen-stimulated spleen cell conditioned medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; DEAE, diethylaminoethyl; HPTLC, high performance thin layer chromatography. Glycosphingolipids are designated according to the recommendation of the Nomenclature Committee of the IUPAC [29] and gangliosides are designated as described [30].

Introduction

Glycosphingolipids have been demonstrated to bind toxins, bacteria, and viruses [1], and to be cell type-specific adhesion ligands for selectins [2–3]. In addition, regulation of cell differentiation, cell growth, and morphogenesis by gangliosides has been reported in several cell systems [4–10].

According to their structures, growth factor receptors are divided into several groups: the tyrosine kinase receptor family, the tumour necrosis factor receptor family, the interferon receptor family, the haematopoietic receptor family, and others. It has been reported that gangliosides and their related compounds are associated with transmembrane signalling involving tyrosine kinase-receptors and receptors for platelet-derived growth factor, epidermal cell growth factor and insulin [11–14]. On the other hand, similar associations of glycosphingolipids with signal transduction involving the other cytokine receptor families have not yet been investigated. Furthermore, even haematopoietic factor-associated glycosphin-

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golipids have not been well characterized for association with receptor family members such as the interleukin-3 (IL-3) receptor. IL-3 is a multi-CSF (colony-stimulating factor) which supports growth and differentiation of haematopoietic stem cells and activates haematopoietic cell function at the terminal differentiation stage. It is of great interest to select the *IL-3* gene as a model for analysis of haematopoietic factor-associated glycolipids and their expression mechanism.

Factor-dependent cell lines have often been utilized for analysing the functions of cytokines and mechanisms of signal transduction. Murine myelogenous leukaemia cell line NFS60 is one such multi-factor-dependent cell line [15]. Using a previously obtained subclone NFS60-I7 that is exclusively dependent on IL-3, we prepared NSF60 sublines transfected with *IL-3* cDNA-containing plasmid for analysing IL-3-associated glycolipids. By using NFS60-I7 and its *IL-3* gene-transfectants, we report here the comparison of the actions of intracellularly expressed IL-3 and exogenous IL-3 on cell growth and on IL-3-associated ganglioside expression. We show that it is not the extracellularly secreted IL-3 but rather the intracellularly generated IL-3 which supports cell growth and IL-3-associated ganglioside expression. The expression of IL-3-associated ganglioside as well as autonomous cell growth may be due to an intracellular autocrine mechanism.

Materials and methods

Materials

The plasmid pcD-MCGF, a pBR322 derivative containing murine *IL-3* cDNA, [16] was kindly provided by Dr K. Arai (Institute of Medical Science, Tokyo University). Restriction endonucleases were obtained from Nippon Gene (Japan) and TAKARA (Japan). A murine recombinant IL-3 (rIL-3) was from Genzyme Corporation (Cambridge, MA, USA). Rabbit anti-mouse IL-3 antiserum was kindly presented by Dr Sudo (Toray Basic Research Institute, Japan). Polyclonal anti-GM1a antibody and anti-asialoganglioside GA1 antibody were purchased from Iatron Laboratories, Inc. (Japan). High-performance thin layer chromatography (HPTLC) plates were obtained from Merck (Darmstadt, Germany). All other reagents were of the highest grade commercially available.

Cells and cell culture

A murine myelogenous leukaemia cell line NFS60-I7 dependent exclusively on exogenous IL-3 was established in our laboratory from the original NFS60 cell line [15], which was generously supplied by Dr J.N. Ihle (Saint Jude Children's Hospital, Memphis, TN). The cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCCM; final concentration of 4–8%). In the experiments, 5 or 100 U ml^{-1} murine rIL-3 was used in place of PWM-SCCM unless otherwise indicated. *IL-3*-gene transfected NFS60 cells were prepared by transfection of pcD-MCGF linearized with *Cla* I using an electroporation technique as described previously [17]. The transfectants were isolated as colonies by culturing the cells in methylcellulose semi-solid medium in the absence of rIL-3 for 14–21 days. The transfectants were examined to be independent of exogenous IL-3 and to show autonomous cell growth.

RNA blot and Southern blot analyses

Total cellular RNA was extracted from the parental and transfected NFS60 cells using the guanidium/CsCl method. RNA blot analysis was carried out as described [18] using the [^{32}P]-labelled 0.6 kb IL-3 specific cDNA fragment. High molecular weight DNA was prepared from the parental cells and the *IL-3* gene-transfectants, and digested with restriction endonucleases, *Pst* I and *Hind* III (Nippon Gene, Tokyo). Southern blot hybridization was performed with a [^{32}P]-labelled 0.6 kb *Pst* I/*Nco* I fragment of the murine IL-3 specific cDNA according to the standard methods [19].

Biological assays for IL-3 activity

IL-3 activities in the conditioned media or in the cellular extracts were measured according to the method of colorimetric MTT assay using proliferation of another IL-3 dependent FDC-P2 cell line [20]. The conditioned media were collected at a cell density of 2×10^6 cells ml^{-1}. FDC-P2 cells were incubated for 24 h at 2×10^5 cells ml^{-1} in 100 μl of RPMI-1640 medium containing 10% FCS in the presence of 10% conditioned media or the cellular extracts.

Assessment of tumorigenicity

The parental and transfected cells ($1–5 \times 10^6$ cells) were injected subcutaneously into 6-week-old Balb/c (nu/nu) nude mice. Mice were observed routinely for 12 weeks.

Analyses of gangliosides

Cells for glycosphingolipid analyses were harvested and stored at −80 °C until use. For glycolipid analysis, the parental cells were cultured in the presence of 8% PWM-SCCM unless otherwise stated. Glycosphingolipids were prepared by a combination of chloroform-methanol extraction and DEAE-Sephadex A-25 column chromatography [21]. After mild alkaline treatment, dialysis, and lyophilization, ganglioside fractions were separated on HPTLC plates with a solvent system of chloroform: methanol:0.2% $CaCl_2$ (50:50:10, v/v/v). The patterns of ganglioside fractions from the parental and the transfected cells were also compared using cells metabolically

labelled with [^{14}C]-Gal followed by extraction, purification, separation by HPTLC, and autoradiography [22].

Immunostaining on HPTLC plate

Immunostaining was performed according to the method described by Magnani *et al.* [23] with slight modification. Briefly, the gangliosides were chromatographed on HPTLC aluminium sheets, treated with sialidase (*Clostridium perfringens*) on the plate, reacted successively with a first antibody, a second rabbit anti-mouse IgM antibody, and [^{125}I]-labelled protein A solution (Amersham), and then autoradiographed.

Results

IL-3 production by IL-3 gene-transfected NFS60 cells

The parental NFS60 cells, that were exclusively dependent on exogenous IL-3, were designated as NFS60-I7. Following transfection of the *IL-3* gene into NFS60-I7 cells and selection of monoclonal transfectants, 10 sublines showing autonomous cell growth in the absence of exogenous IL-3 were established. The transfectants secreted various amounts of IL-3 into conditioned media as shown in Fig. 1 and were divided into three groups according to their IL-3 producing capabilities. High producing cells secreting IL-3 at a range from 100 to 280 U ml^{-1} were designated NFS60-H7, -H1, -H4, and -H10; intermediate cells secreting from 10 to 100 U ml^{-1} were designated NFS60-M2, -M3, and -M6; and low producers with less than 10 U ml^{-1} were designated NFS60-L5, -L8, and -L9. In spite of their different IL-3 producing capabilities, the cell lines did not differ in growth rates (data not shown). Activity of intracellularly expressed IL-3 was measured by the colorimetric MTT assay using FDC-P2 cells. High IL-3 producer NFS60-H7 cells showed an intracellular activity of 55 U per 10^7 cells, while low IL-3 secretor NFS60-L5 cells exhibited an activity of 12 U per 10^7 cells. In contrast to the extracellular IL-3 production, the intracellular activities did not show a great difference between the high and low IL-3 producing sublines. In subsequent experiments, we studied mainly a clone designated NFS60-H7 that produced extracellularly the highest IL-3 activity and a clone NFS60-L5 that was one of the lowest IL-3-secretors into the conditioned media among the established sublines.

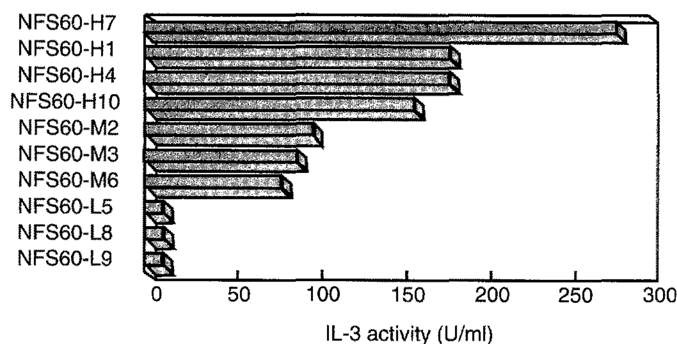

Figure 1. IL-3 activity of the various *IL-3* gene-transfected cell lines. IL-3 activity produced in the conditioned media of the indicated cell lines was measured according to the colorimetric MTT assay using FDC-P2 cells as described in the text. FDC-P2 cells (2×10^4) were incubated for 24 h in 100 μl RPMI-1640 medium containing 10% FCS in the presence of 10% the conditioned medium of each *IL-3*-gene transfectants.

Expression and integration of transfected IL-3 gene in NFS60 cells

We characterized the transfected NFS60-H7 and NFS60-L5 cells by RNA and Southern blot analyses. RNA blot analysis revealed that the parental NFS60-I7 cells did not express the *IL-3* message (Fig. 2A, left lane). By contrast, *IL-3* gene transfected NFS60-H7 cells expressed significant amounts of the transcripts (right lane). Although NFS60-L5 cells expressed an *IL-3* message of the same size, the amount was lower than that of NFS60-H7 cells (data not shown). Southern blot analysis using *Pst*I-digested DNA from the transfected NFS60-L5 and NFS60-H7 cells showed that a new 3.0 kb band in addition to those found in the parental NFS60-I7 cells (1.8, 1.3, and 0.8 kb) was exhibited (Fig. 2C). This additional 3.0 kb band was derived from the transfected plasmid pcD-MCGF that has 2 *Pst*I restriction sites 3.0 kb apart. The same analyses using *Hind*III-digested DNA showed two additional bands, 4.8 and 3.5 kb (data not shown). Consequently, it appears that the exogenous *IL-3* gene was successfully integrated in chromosomes and expressed constitutively.

Tumorigenicity of IL-3 gene-transfected NFS60 cells

Subsequently, the tumorigenicity of *IL-3* gene-transfected NFS60 cells was assessed by injecting cells into nude mice. As summarized in Table 1, NFS60-I7 cells as well as the vehicle only (PBS) did not cause any tumours – at least for 12 weeks. In contrast, the low IL-3-producer NFS60-L5 and high IL-3-secretor NFS60-H7 cells generated subcutaneous tumours in all mice independent of the number of cells injected (Table 1). Moreover, infiltration of leukaemic cells into spleen and liver was observed in the mice injected with the transfected cells. However, sizes of tumour, spleen, and liver did not show significant differences between the NFS60-L5 and NFS60-H7 cells (data not shown).

Analyses of cell growth potential in IL-3 gene-transfected NFS60 cells

To examine the relationship between IL-3 production and cell growth potential, we conducted MTT colorimetric experiments for cell growth of NFS60 cells in the presence of rIL-3 and/or rabbit anti-mouse IL-3 antibody.

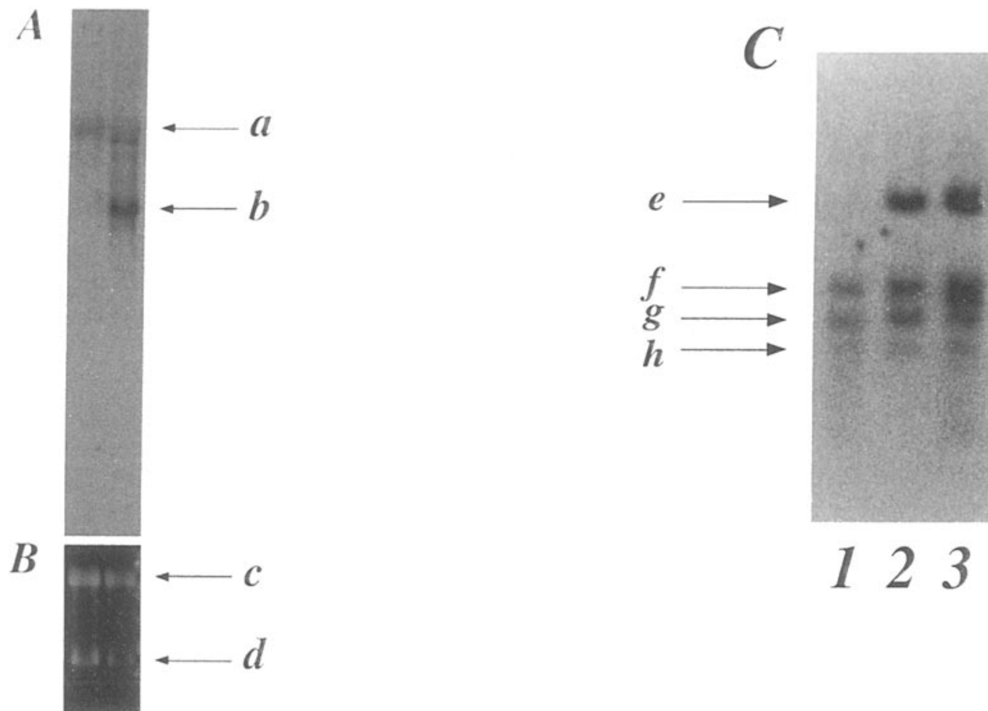

Figure 2. Northern and Southern blot analyses of the IL-3 dependent parental cells and *IL-3* gene transfected cells. (A) Total cellular RNA was extracted using the guanidium/CsCl method. Each 20 μg of total RNA from NFS60-I7 (left lane) and NFS60-H7 (right lane) cells was electrophoresed in 0.75% agarose gel, blotted to nylon membrane, Biodyne B (Nippon Genetics, Tokyo, Japan), hybridized with [^{32}P]-labelled murine *IL-3* specific cDNA *Nco* I/*Pst* I fragment, and detected with autoradiography. Arrows a and b indicate the positions of non-specific 28S band and specific *IL-3* message, respectively. (B) Each corresponding ribosomal RNA was stained with ethidium bromide before transfer to the membrane. Arrows c and d indicate the positions of 28S and 18S ribosomal RNA band, respectively. (C) High molecular weight DNA was prepared from NFS60-I7 (lane 1), NFS60-L5 (lane 2), and NFS60-H7 cells (lane 3), and digested with restriction endonuclease *Pst* I. The DNA preparations were subjected to 1% agarose electrophoresis, transferred to nylon membrane, Hybond-N$^+$ (Amersham Inc., Tokyo), hybridized with [^{32}P]-labelled murine *IL-3* specific cDNA *Nco* I/*Pst* I fragment, and detected by autoradiography. Arrows e, f, g, and h indicate the positions of 3.0, 1.8, 1.3, and 0.8 kb, respectively.

Table 1. Tumorigenicity of NFS60 cell lines. Cells were washed with PBS, suspended in 0.2 ml PBS, and injected subcutaneously into balb/c nude mice (nu/nu). The mice were observed for 12 weeks.

Cell line	*Number of cells*	*Tumour bearing mice/ injected mice*
PBS	(–)	0/2
NFS60-I7	1×10^6	0/3
NFS60-I7	5×10^6	0/3
NFS60-L5	1×10^6	3/3
NFS60-L5	5×10^6	3/3
NFS60-H7	1×10^6	3/3
NFS60-H7	5×10^6	3/3

As shown in Table 2 (the first two columns), the low IL-3-producer NFS60-L5 cells did not show a significant increase in growth potential even in the presence of 100 U ml^{-1} rIL-3. In addition, while anti-IL-3 antibody abolished significantly the growth potential of IL-3 dependent parental NFS60-I7 cells (Table 2, fourth column), the antibody could not suppress growth of the transfectants NFS60-L5 and NFS60-H7 in the absence or presence of rIL-3 (Table 2, third and fourth columns). Although it seemed that anti-IL-3 antibody potentiated the growth of the transfected cells in the absence of rIL-3 (Table 2, first and third columns), this was not statistically significant. These experiments indicate that the autonomous growth potential of the *IL-3* transfectants was not based upon the extracellular IL-3 secreted by the cells.

Table 2. Effect of rabbit anti-IL-3 antibody on cell growth of NFS60 cell lines. NFS60-I7, NFS60-L5, and NFS60-H7 cells were cultured in the presence or absence of exogenous rIL-3 and/or rabbit anti-IL-3 antibody for 24 h. The cell growth was measured by MTT colorimetric assay.

Cells	*rIL-3 (–)*	*rIL-3 (+)*[a]	*rIL-3 (–) + anti-IL-3 Ab*[b]	*rIL-3 (+)*[c] *+ anti-IL-3 Ab*[b]
NFS60-I7	ND[d]	100.0 ± 9.9[e]	ND[d]	8.6 ± 2.3[e]
NFS60-L5	77.0 ± 9.4[e]	80.2 ± 10.6[e]	88.3 ± 8.0[e]	83.5 ± 10.8[e]
NFS60-H7	78.6 ± 12.0[e]	77.6 ± 8.3[e]	102.0 ± 14.2[e]	98.1 ± 9.0[e]

[a]Concentration of rIL-3 was 100 U ml^{-1}.
[b]Rabbit anti-mouse IL-3 antibody was used at 10% concentration in the culture medium.
[c]Concentration of rIL-3 was 5 U ml^{-1}.
[d]Not done.
[e]Values are expressed as relative MTT activities when that of NFS60-I7 cells with rIL-3 only is calculated as 100. Values represent means of the triplicate ±SE.

Analyses of gangliosides from IL-3 gene-transfected NFS60 cells

To explore the potential cytokine-associated glycosphingolipids, we prepared ganglioside fractions from the parental and transfected NFS60 cells and analysed them by HPTLC (Fig. 3). In the IL-3 dependent NFS60-I7 cells, there were two major components (lane 2, arrows f and h, designated G_{X1} and G_{X2}). G_{X1} co-migrated with GM1a and G_{X2} migrated between GD1a and GD1b. In the transfectant NFS60-L5 and NFS60-H7 cells, however, a component co-migrating with GD1a was significantly expressed (lane 3 and 4, arrow g, designated G_{XN}), while G_{X1} decreased in expression. Although G_{X1} seemed to be a minor component in *IL-3* gene-transfected cells as shown in Fig. 3, this was due to the difference in harvesting time between the parental and transfected cells. The relative amount of G_{X1} varied from the time of harvest both in the parental and transfected cells. NFS60-L5 and NFS60-H7 cells expressed almost the same significant amount of G_{X1} as in NFS60-I7 cells, when the cells were harvested at the mid-logarithmic stage (Table 3, first three columns). The structure of G_{XN} was estimated by the technique of neuraminidase (*C. perfringens*) treatment on the HPTLC plate followed by staining with antibodies against asialoganglioside GA1 and GM1a. G_{XN} treated by *C. perfringens* neuraminidase was not detected by an anti-GA1 antibody but by a specific antibody against GM1a (data not shown). This means G_{XN} has a II^3NeuAc-$GgOse_4$Cer (GM1a) backbone. As G_{XN} co-migrated with GD1a, G_{XN} was concluded to be IV^3NeuAc,II^3NeuAc-$GgOse_4$Cer (GD1a).

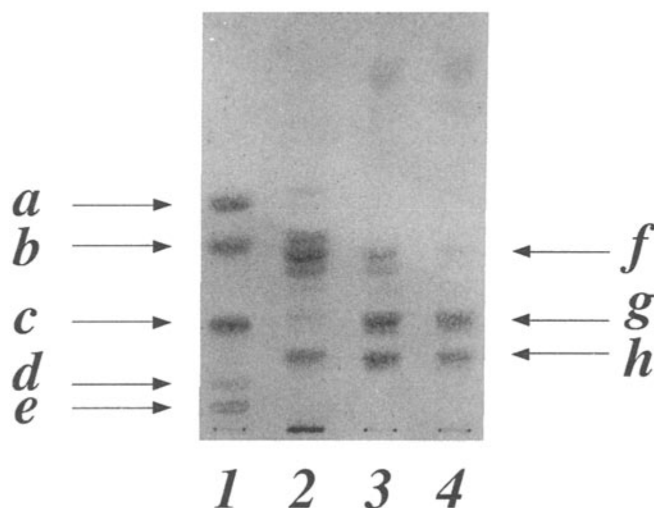

Figure 3. Ganglioside analysis from the IL-3 dependent parental cells and *IL-3* gene-transfected cells. Gangliosides from NFS60-I7 (lane 2), NFS60-L5 (lane 3), and NFS60-H7 (lane 4) cells were chromatographed in chloroform:methanol:0.5% aqueous $CaCl_2$ (50:50:10, v/v/v), and detected by resorcinol-hydrochloric acid spray. The authentic gangliosides were developed in lane 1; a: GM2, b: GM1a, c: GD1a, d: GD1b, and e: GT1b. Arrows f, g, and h indicate G_{X1}, G_{XN}, and G_{X2}, respectively.

Effect of exogenous IL-3 and anti-IL-3 antibody on ganglioside expression in NFS60 cells

To examine the relationship between IL-3 production, effect of exogenous IL-3, and cell growth capability, we analysed the metabolically labelled gangliosides from the IL-3 dependent parental and *IL-3* gene-transfected cells co-cultured with exogenous IL-3 and/or rabbit anti-mouse IL-3 antibody. The amounts of GD1a were the same in spite of variations in IL-3 producing capability in the *IL-3* gene-transfected NFS60-L5 and NFS60-H7 cells (Table 3, the second and third columns). Subsequently, the effect of exogenous IL-3 on GD1a expression in IL-3 dependent NFS60-I7 cells was investigated. As shown in Table 3 (the first and fourth columns), GD1a was expressed in NFS60-I7 cells treated with 100 U ml^{-1} rIL-3 at a level five times greater than in NFS60-I7 cells treated with 8% PWM-

Table 3. Effect of exogenous rIL-3 and rabbit anti-IL-3 antibody on ganglioside expression in NFS60 cell lines. NFS60-I7, NFS60-L5, and NFS60-H7 cells were labelled metabolically with [^{14}C]Gal in the presence or absence of 8% PWM-SCCM and/or 10% rabbit anti-IL-3 antibody for 24 h. Ganglioside fractions from NFS60 cells were prepared, developed on HPTLC using solvent system of chloroform:methanol:0.2% $CaCl_2$ (50:50:10, v/v/v), visualized by autoradiography, and quantitated by Shimadzu CS-9000.

Gangliosides[a]	*NFS60-I7 8% PWM-SCCM*	*NFS60-L5*	*NFS60-H7*	*NFS60-I7 100 U ml^{-1} rIL-3*	*NFS60-H7 rabbit anti-IL-3 Ab*
G_{X1}	5.85	6.47	6.94	8.61	6.90
G_{XN}(GD1a)	0.32	2.17	2.16	1.65	2.14
G_{X2}	1.97	1.54	1.87	2.10	1.54

[a]Values are expressed as μg of each ganglioside component per 1×10^7 cells. Ganglioside concentrations were estimated by [^{14}C] radioactivities and multiplied by the relative intensity obtained from the analyses using CS-9000. The results presented in this Table are from one set of determination out of two independent assays using replicate cell cultures.

SCCM (this was equivalent to 5 U ml^{-1}). Because of IL-3-dependency, NFS60-I7 cells do not grow well in the presence of anti-IL-3 antibody and we could not obtain sufficient cells treated with rIL-3 in the presence of the antibody (see Table 2, fourth column). In NFS60-H7 cells, however, anti-IL-3 antibody could not decrease the amount of GD1a (Table 3, third and fifth columns). These results revealed that GD1a was generated by the intracellularly expressed IL-3, that the amount of expressed GD1a was also controlled by the amount of IL-3 added extracellularly to the parental NFS60-I7 cells, and that GD1a was not abolished in its expression by the effect of anti-IL-3 antibody.

Discussion

In the present study, we selected an IL-3 dependent cell line NFS60-I7 and *IL-3* gene as a model of analysing haematopoietic factor-associated glycolipids. By *IL-3* gene transfection into NFS60-I7, the *IL-3* cDNA was presumably integrated into chromosomes and expressed constitutively while the endogenous *IL-3* gene remained unexpressed in the transfected NFS60-H7 and NFS60-L5 cells. The transfected cell lines acquired autonomous cell growth without exogenous IL-3, and secreted extracellularly various amounts of IL-3 ranging from 10 to 280 U ml^{-1}. IL-3 production to the culture medium was shown to correlate with expression level of *IL-3* message. On the other hand, autonomous cell growth potential was revealed to have little connection with extracellular IL-3 production, since additional exogenous IL-3 did not potentiate the growth rate of low IL-3-producer, NFS60-L5 cells. In addition, tumorigenicity was also revealed to have little connection with extracellular IL-3 production

Analysing and comparing gangliosides of *IL-3* gene-transfected NFS60-H7 cells with the IL-3 dependent parental NFS60-I7 cells, we found an IL-3-associated ganglioside pattern in NFS60-H7. Although the relative abundance of each component seems to vary from mid-logarithmic stage to late-logarithmic phase, the major components were G_{X1} and G_{X2} in NFS60-I7 cells. However, the third major component G_{XN} was dramatically expressed in NFS60-H7 cells. By using HPTLC and immunostaining with anti GgOse4Cer and anti GM1a antibodies after *C. perfringens* neuraminidase treatment, G_{XN} was concluded to be IV^{3}NeuAc,II^{3}NewAc-GgOse$_4$-Cer (GD1a). The precise structural analyses will be presented and discussed elsewhere (A. Tsunoda, M. Nakamura, *et al.*, unpublished data). It would be of great interest to see the effect of anti-GD1a antibody on NFS60 cells. Unfortunately, however, G_{X1} and G_{X2} have the same sugar structures as GD1a at the non-reducing termini and the same core structures as GD1a (i.e. GA1 structure) (A. Tsunoda, M. Nakamura, *et al.*, unpublished data). Further, to our knowledge GD1a-specific antibody has not been generated so far. Anyhow, it should be clarified whether there might be some interactions between cell surface gangliosides and cell growth machinery in NFS60 cells.

Although autonomous cell growth potential was revealed to have little connection with extracellular IL-3 producing capability, it was striking that anti-IL-3 antiserum could not neutralize the growth of the transfectant NFS60-H7 and NFS60-L5 cells. From these findings, we postulate that it is not the secreted IL-3 but the intracellular IL-3 produced in the cells that is critical for the autonomous cell growth of the *IL-3* gene-transfected NFS60 cells. The introduction of the *GM-CSF* gene into GM-CSF dependent FDC-P1 cells was reported to result in their autonomous growth even in the presence of the antiserum [24]. Futhermore, COOH-terminal modified IL-3 was introduced in the IL-3 dependent cells and shown to be retained intracellularly and to stimulate autonomous cell growth [25].

It was demonstrated that exogenous rIL-3 induced ganglioside GD1a expression in the parental NFS60-I7 cells. The intensity of GD1a in the rIL-3-treated NFS60-I7 cells was less than that from NFS60-H7 cells. However, at least 100 U ml^{-1} of rIL-3 would be enough for the GD1a expression in NFS60-I7 cells. Although extracellular IL-3 was revealed to induce IL-3-associated

ganglioside GD1a expression in NFS60-I7 cells, the low IL-3 producing transfectant NFS60-L5 and high IL-3 secreting NFS60-H7 cells expressed almost equal amounts of GD1a. Thus, ganglioside GD1a expression was shown to have little correlation with IL-3 production. In addition, exogenous anti-IL-3 antiserum could not neutralize GD1a expression. These results indicated that is it not the secreted IL-3 but the intracellular IL-3 produced inside the cells that is critical for GD1a expression in the *IL-3* gene-transfected NFS60 cells. Our results strongly suggested that autocrine growth can occur as a result of the intracellular action of the growth factor and that autocrine growth factor might act independently of its normal receptor in the transfectants.

It has been reported that gangliosides and their related compounds are associated with transmembrane signalling of tyrosine kinase-receptor family members. However, similar associations of glycosphingolipids with signal transduction through the haemotopoietic receptor families have not yet been well investigated. Even haematopoietic factor-associated glycosphingolipids have not been analysed and characterized. Our present report proposes that GD1a is an IL-3-associated ganglioside and is expressed in a tightly coupled manner with autonomous cell growth. GD1a has been reported as a differentiation marker of murine T helper lymphocyte subpopulation T_H2 by Ebel *et al.* [26]. The functional role of these subtype specific gangliosides was suggested to be involved in cell-cell interactions and homing processes as reported in other systems [27–28], or an association with the cellular activity of the T_H2 cells. Together with our current report, these data suggest that GD1a may play an important role in murine immune and haematopoietic cells. Cell surface gangliosides are also considered to be modulators of the activity of functional membranous proteins. Interactions of cell surface gangliosides with the family of tyrosine-kinase receptors have been described [11–14]. However, similar associations of glycosphingolipids with signal transduction involving the other cytokine receptor families have not yet been well investigated. Whether GD1a plays any functional role in the activity of IL-3- or IL-3 receptor-related proteins (e.g. IL-3 signalling) in NFS60 cells remains to be elucidated.

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