

Ganglioside GQ1b enhances Ig production by human PBMCs

Naoko Kanda, MD, PhD, and Kunihiko Tamaki, MD, PhD Tokyo, Japan

Background: Gangliosides are glycosphingolipids that contain sialic acid and have various immunomodulatory effects.

Objective: We studied in vitro effects of gangliosides on human humoral immune responses.

Methods: PBMCs from healthy volunteers were cultured with gangliosides GM1, GM2, GM3, GD1a, GD1b, GD2, GD3, GT1b, and GQ1b. The amounts of IgG, IgM, and IgA and cytokine activity in the culture supernatants were measured with use of an ELISA. Proliferation was determined by [³H] thymidine uptake.

Results: Among the various gangliosides tested, GQ1b most strongly enhanced spontaneous IgG, IgM, and IgA production by human PBMCs. The effect of GQ1b was revealed at 0.1 μmol/L, increased dose dependently, and was maximized at 10 μmol/L. Weaker but significant stimulatory effects on the Ig production were manifested by GM2 and GD1a, whereas GT1b and GD1b were inhibitory. None of the gangliosides examined affected the proliferation of PBMCs. GQ1b did not enhance Ig production of B cells alone. Anti-IL-6 and anti-IL-10 antibody each partially blocked GQ1b-induced enhancement of the Ig production by PBMCs, and the addition of both antibodies completely blocked the enhancement. GQ1b enhanced both IL-6 and IL-10 production of T cells without affecting those of monocytes or B cells. GQ1b enhanced Ig production of T and B cell culture without monocytes. When T cells were preincubated with GQ1b, washed and added to B cells and monocytes, and then cultured together, the Ig production was also enhanced, although to a lesser extent than the whole time incubation. Exogenous IL-6 and IL-10 each enhanced Ig production of B cells alone, and the addition of both gave additive effects. **Conclusion:** These results suggest that GQ1b may indirectly enhance Ig production of B cells by promoting IL-6 and IL-10 production of T cells in whole PBMC culture. That GQ1b may act as an important immunostimulator is also indicated. (*J Allergy Clin Immunol* 1998;102:813-820.)

Key words: Ganglioside, GQ1b, immunoglobulin, T cell, IL-6, IL-10

Gangliosides* are glycosphingolipids that contain sialic acid. They are expressed on the plasma membranes of various cells, shed into the extracellular environment, and exhibit various physiologic functions.¹⁻⁴ Previous studies reported that various gangliosides either stimulated or inhibited cellular immune responses in mice and in

Abbreviations used

CNS: Central nervous system
³H-TdR: [³H] thymidine
SAC: *Staphylococcus aureus* Cowan strain I

humans.^{2,5-7} For example, GM1, GD1a, and GD1b inhibited lipopolysaccharide-induced proliferation of murine splenocytes, whereas GM2 did not.⁷ GM2 and GD2 enhanced IL-2-driven proliferation of T cells from patients with melanoma, whereas GM3 and GD3 were inhibitory.² Several studies also reported the effects of gangliosides on human⁸⁻¹¹ and murine^{12,13} humoral immune responses. However, the results of these studies are inconsistent with one another; GM1 enhanced IgG, IgM, IgA, and IgE production of human plasma cells,⁸ whereas it suppressed anti-sheep erythrocyte plaque-forming cell responses of murine splenocytes.^{12,13} GM2 and GM3 suppressed IgG4 and IgE¹⁰ production of IL-4 or IL-13 plus hydrocortisone-activated human tonsillar small resting B cells, whereas they had no effect on Ig production of human plasma cells.⁸ Presumably the effects of individual gangliosides may vary with species, types of Ig-producing cells, and mitogens or cytokines used for activation. The previous investigators⁸⁻¹³ emphasized the direct effects of gangliosides on Ig-producing cells but did not mention whether gangliosides affect the helper functions of accessory cells. Because T cells and monocytes sustain Ig production of B cells by releasing cytokines and/or direct contact with B cells,¹⁴ elucidating the effects of gangliosides on the functions of accessory cells is very important. Besides, the previous investigators mostly used neoplastic or immortalized cells such as lymphoblastoid cell lines or mitogen- or cytokine-driven systems for Ig production and thus did not examine the effects of gangliosides on constitutive Ig production by nonimmortalized cells.

We herein examined the in vitro effects of various gangliosides on spontaneous Ig production by normal PBMCs. Among the gangliosides tested, GQ1b revealed the most stimulatory effect. We then focused on GQ1b and further examined the mechanism for its stimulatory effect, that is, whether GQ1b acts on B cells directly or enhances accessory cell functions to help their Ig production, or both.

*The nomenclature for gangliosides used in this article follows the system of Svennerholm.⁴²

From the Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan.

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Reprint requests: Naoko Kanda, MD, PhD, Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan.

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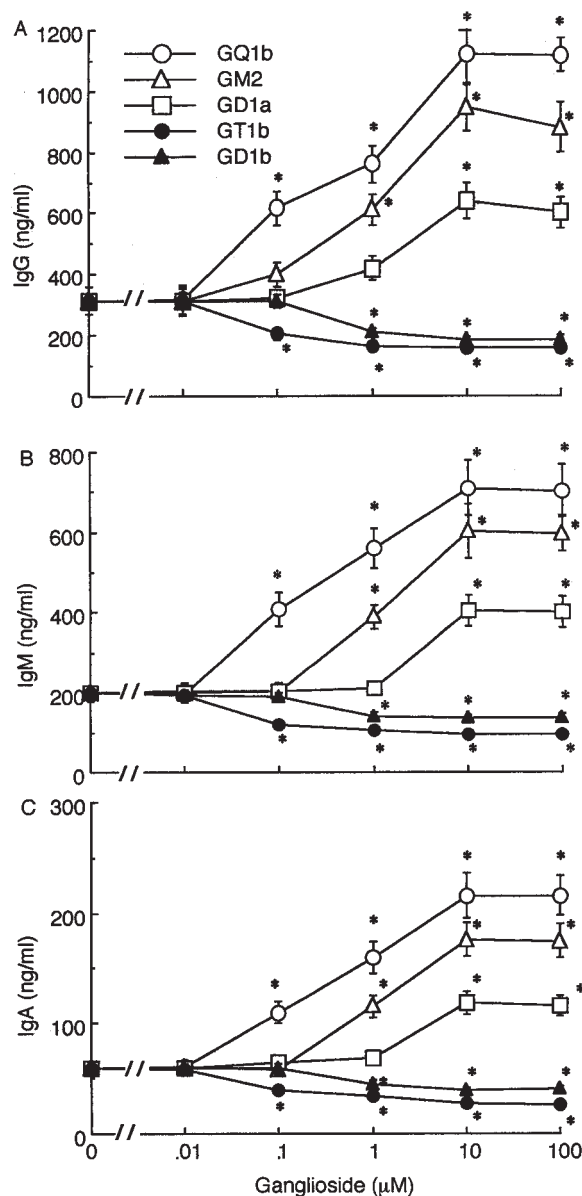


FIG 1. Dose dependency of the effects of various gangliosides on Ig production by human PBMCs. PBMCs (2×10^5 cells/200 μ L/well) from 1 healthy donor were cultured in triplicate for 5 days in the presence or absence of indicated doses of gangliosides, GQ1b, GM2, GD1a, GD1b, and GT1b. The culture supernatants were assayed for IgG (A), IgM (B), and IgA (C) by ELISA. Values are the mean $\pm$ SD of triplicate cultures. * $P < .05$ versus control cultures. The data represent 6 separate experiments using PBMCs from 6 different donors.

METHODS

Reagents

Highly purified bovine brain gangliosides GM1, GM2, GM3, GD1a, GD1b, GD2, GD3, GT1b, and GQ1b were purchased from Sigma (St Louis, Mo). Monoclonal mouse IgG anti-human IL-2, IL-4, and IL-6 antibodies and recombinant human IL-6 were purchased from Boehringer Mannheim (Indianapolis, Ind). Monoclonal mouse IgG anti-human IL-1 α and IL-1 β antibodies were obtained from

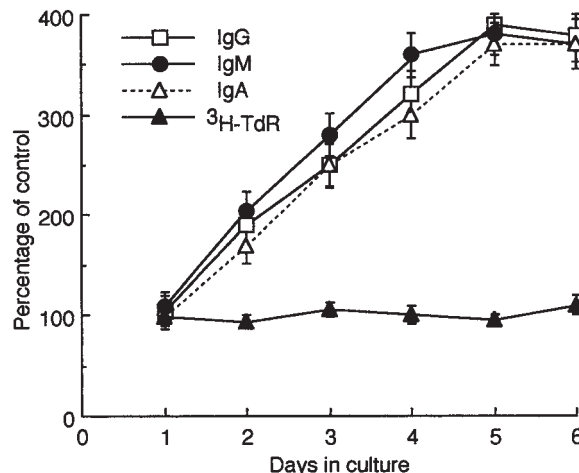


FIG 2. Kinetics of the effects of GQ1b. PBMCs (2×10^5 cells/200 μ L/well) from 6 different donors were cultured in triplicate in the presence of GQ1b at 10 μ mol/L for 1 to 6 days. IgG, IgM, and IgA production and ³H-TdR uptake were evaluated on each day as described in the Methods section. Results are expressed as the percentage versus control cultures without GQ1b. Bars show the mean $\pm$ SD ($n = 6$). SDs of triplicate cultures from individual donors were $<10\%$ of the means. The mean amounts of controls ($n = 6$) on days 1, 2, 3, 4, 5, and 6 were 120, 150, 182, 270, 310, and 324 ng/mL in IgG, 98, 113, 146, 180, 206, and 211 ng/mL in IgM, 11, 18, 23, 35, 49, and 62 ng/mL in IgA, and 2147, 2764, 3845, 4763, 5012, and 4756 cpm in ³H-TdR uptake, respectively.

Immunotech (Westbrook, Me). Monoclonal mouse IgG anti-human IL-10 antibody and recombinant human IL-10 were obtained from R&D Systems (Minneapolis, Minn). Monoclonal mouse IgG anti-GQ1b antibody 7F5 was obtained from Mectro (Tokyo, Japan). Control mouse IgG was purchased from DAKO (Tokyo, Japan).

Preparation of PBMCs, monocytes, B cells, and T cells

Blood was taken from healthy volunteers who were informed of the objectives and methods of this study and consented to participate. PBMCs were isolated by centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) as described.¹⁵ PBMCs were allowed to adhere to plastic dishes. From the dish-adherent cells, CD3⁺, CD19⁺, and CD56⁺ population was isolated by repeating negative selection using immunomagnetic beads (Dynal, Great Neck, NY) as described¹⁶ and was used as monocytes. This monocyte population was $>97\%$ CD14⁺, and the contamination of CD3⁺, CD19⁺, or CD56⁺ cells was $<1\%$, which was determined by flow cytometry. From the nonadherent cells, CD56⁺ cells were isolated by the immunomagnetic negative selection and were incubated with neuraminidase-treated sheep erythrocytes as described.¹⁷ From the rosette-forming cells, CD14⁺ and CD19⁺ population was isolated by the immunomagnetic negative selection and was used as T cells. This T-cell population was $>98\%$ CD3⁺, and the contamination of CD14⁺, CD19⁺, or CD56⁺ cells was $<2\%$. From the non-rosette-forming cells, CD3⁺ and CD14⁺ population was isolated by the immunomagnetic negative selection and was used as B cells. This B-cell population was $>97\%$ CD19⁺, and the contamination of CD3⁺, CD14⁺, or CD56⁺ cells in this population was $<1\%$.

Cell culture

PBMC or B cells (2×10^5 cells/200 μ L/well) were cultured in triplicate in round bottom 96-well tissue culture plates (Costar,

TABLE I. The effects of GQ1b on cytokine production by PBMCs

Conditions	Cytokine production (pg/mL)					
	IL-1 α	IL-1 β	IL-2	IL-4	IL-6	IL-10
Control	18 $\pm$ 5	22 $\pm$ 2	28 $\pm$ 8	10 $\pm$ 3	258 $\pm$ 36	156 $\pm$ 27
GQ1b	18 $\pm$ 4	22 $\pm$ 2	28 $\pm$ 9	11 $\pm$ 4	531 $\pm$ 72*	329 $\pm$ 56*

PBMCs (2×10^5 cells/200 μ L/well) were cultured in triplicate in the presence or absence of GQ1b (10 μ mol/L) for 24 hours, and the culture supernatants were analyzed for cytokines by ELISA. Values are the mean $\pm$ SD (n = 6). SDs of triplicate cultures from individual donors were <10% of the means. * P < .05 versus control cultures.

TABLE II. The effects of IL-6 and IL-10 on Ig production and proliferation of B cells

Conditions	Ig production (ng/mL)			³ H-TdR (cpm)
	IgG	IgM	IgA	
Control	50 $\pm$ 6	35 $\pm$ 2	10 $\pm$ 1	2818 $\pm$ 161
IL-6	140 $\pm$ 13 (180)*	85 $\pm$ 6 (143)*	22 $\pm$ 1 (120)*	2815 $\pm$ 186 (0)
IL-10	110 $\pm$ 9 (120)*	75 $\pm$ 6 (114)*	25 $\pm$ 1 (150)*	6250 $\pm$ 494 (122)*
IL-6 + IL-10	200 $\pm$ 14 (300)*†‡	126 $\pm$ 11 (260)*†‡	37 $\pm$ 3 (270)*†‡	6375 $\pm$ 494 (126)*†

B cells (2×10^5 cells/200 μ L/well) from 6 different donors were cultured in triplicate with or without IL-6 (20 U/mL) and/or IL-10 (20 U/mL). Ig production was measured on day 5 by ELISA, and ³H-TdR uptake was measured on day 4. Values are the mean $\pm$ SD (n = 6). SDs of triplicate cultures from individual donors were <10% of the means. Values in parentheses are percentage increase versus control cultures.

* P < .0001 versus control cultures.

† P < .001 versus cultures with IL-6 alone.

‡ P < .001 versus cultures with IL-10 alone.

Cambridge, Mass) with or without gangliosides at indicated doses in mycoplasma, endotoxin, and serum-free Hymedium 920 (Kohjin Bio, Tokyo, Japan) at 37° C in an atmosphere of 5% CO₂ in air for 5 days (unless otherwise indicated). The composition of amino acids, minerals, or vitamins in Hymedium 920 is similar to that in RPMI 1640 (Life Technologies, Grand Island, NY), except that the former medium contains 0.48 mg/L thymidine, 2 mg/L human transferrin, 0.2 mg/L 3, 3', 5-triiodo-L-thyronine, 250 mg/L human albumin, and 60 mg/L kanamycin. Gangliosides were dissolved in absolute ethanol and diluted at least 1000-fold to the required concentration in the final medium. Control cultures contained ethanol at the highest concentration used in the experimental cultures, and this level of ethanol was not toxic to PBMCs. The culture supernatants were then harvested and stored at -70° C until use. The amounts of IgG, IgM, and IgA in the supernatants were measured by an ELISA as described.¹⁸ For the measurement of proliferation, PBMCs or B cells were cultured as previously described for 4 days (unless otherwise indicated) and pulsed with 1 μ Ci ³H-TdR for 8 hours. Cells were then harvested and ³H-TdR uptake was counted as described.¹⁹ Proliferation was also determined by a colorimetric assay using sodium 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate as described.²⁰ For the measurement of cytokine production, PBMCs, monocytes, T cells, or B cells (2×10^5 cells/200 μ L/well) were cultured as described previously for 24 hours. The activity of IL-1 α , IL-1 β , IL-2, IL-4, IL-6, and IL-10 in the culture supernatants was measured by ELISA kits (Endogen, Cambridge, Mass) according to the manufacturer's instructions. The lower limits of detection for IL-1 α , IL-1 β , IL-2, IL-4, IL-6, and IL-10 were 2, 1, 6, 2, 1, and 3 pg/mL, respectively.

Statistical analysis

For the data in Figs 1 and 4, one-way analysis of variance with Dunnett's multiple comparison test was used. For the data in Fig 5 and Table I, Wilcoxon signed rank test was used. For the data in Fig 3 and Table II, one-way analysis of variance with Scheffe's multiple comparison test was used. A value of P < .05 was considered significant.

RESULTS

Differential effects of various gangliosides on Ig production by PBMCs

First, various gangliosides were added to the culture medium, and their effects were examined on spontaneous Ig production by human PBMCs. GQ1b, GM2, and GD1a significantly enhanced and GT1b and GD1b inhibited IgG (Fig 1, A), IgM (Fig 1, B), and IgA (Fig 1, C) production. Among these gangliosides, the stimulatory effect of GQ1b was the most strong and was revealed at 0.1 μ mol/L, increased dose dependently, and was maximized at 10 μ mol/L. Ten μ mol/L also appeared to be an optimal concentration for the effects of the other gangliosides; GQ1b (10 μ mol/L) elevated IgG production up to 358% of control, followed by the same concentration of GM2 (303%) and GD1a (205%), whereas GT1b (50%) and GD1b (60%) were inhibitory. Similarly, 10 μ mol/L of GQ1b, GM2, and GD1a elevated IgM production up to 355%, 301%, and 203% of control, respectively, and also elevated that of IgA up to 360%, 293%, and 198%, respectively. In contrast, 10 μ mol/L of GT1b and GD1b reduced IgM production to 48% and 68% of control and that of IgA to 47% and 67%, respectively. Significant stimulatory or inhibitory effects were not revealed by GM1, GM3, GD2, and GD3 (data not shown). Then the effects of gangliosides on the proliferation were examined. None of the gangliosides at 10 μ mol/L affected the proliferation of PBMCs; ³H-TdR uptake in culture with medium alone, GQ1b, GM2, GD1a, GD1b, GT1b, GM1, GM3, GD2, and GD3 were mean $\pm$ SD 5902 $\pm$ 753, 5551 $\pm$ 503, 6002 $\pm$ 376, 6150 $\pm$ 503, 5650 $\pm$ 605, 6153 $\pm$ 541, 5962 $\pm$ 482, 5631 $\pm$ 603, 6204 $\pm$ 511, and 5761 $\pm$ 512 cpm, respectively (n = 6). These values were not signifi-

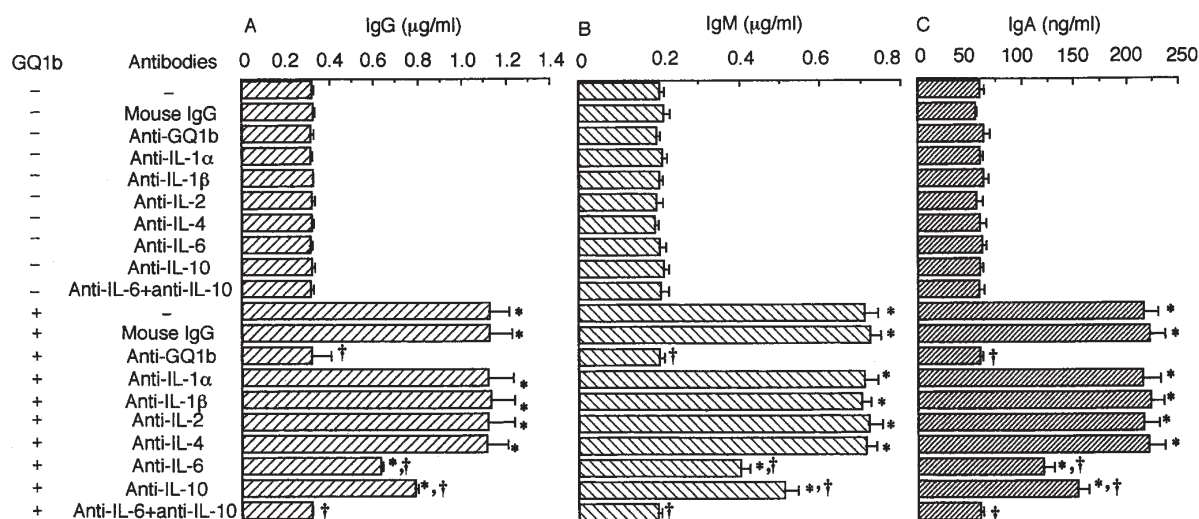


FIG 3. Inhibition of the GQ1b effects by various antibodies. PBMCs (2×10^5 cells/200 μ L/well) from 6 different donors were cultured in triplicate for 5 days with (+) or without (-) GQ1b (10 μ M/L) in the presence or absence of various antibodies (each 10 μ g/mL). IgG (A), IgM (B), and IgA (C) production were measured in each culture as described in the legend for Fig. 1. Bars show the mean $\pm$ SD ($n = 6$). SDs of triplicate cultures from individual donors were $<10\%$ of the means. * $P < .001$ versus control cultures. † $P < .001$ versus cultures with GQ1b alone.

cantly different from one another ($P > .8$, by one-way analysis of variance with Scheffe's multiple comparison test). Although the ganglioside concentration was variously changed from 0.01 to 100 μ M/L, ^{3} H-TdR uptake of PBMCs was not influenced by any ganglioside tested (data not shown). A colorimetric assay also showed similar results (data not shown). Moreover, none of the gangliosides within this concentration range affected the viability of PBMCs; the cell viability was always 92% to 97% of that before culture as examined by a trypan blue exclusion test (data not shown). These results suggest that gangliosides previously described may stimulate or inhibit Ig production of human PBMCs without affecting the proliferation or viability. Because the stimulatory effect of GQ1b was the most remarkable among these gangliosides, the mechanism for its effect was further studied in the following experiments. The precise studies of the other gangliosides, GM2, GD1a, GT1b, and GD1b, will be described elsewhere (papers in preparation).

Kinetics of the stimulatory effect of GQ1b

As shown in Fig 2, GQ1b-induced increase of IgG, IgM, and IgA production by PBMCs was detected on day 2, with the greatest enhancement occurring on day 5. In contrast, GQ1b did not affect ^{3} H-TdR uptake of PBMCs on any day examined. A colorimetric assay also showed similar results (data not shown).

The direct effect of GQ1b on B cells

To examine the direct effect of GQ1b on spontaneous Ig production by B cells, B cells separated from PBMCs

were used. Because it was anticipated that normal B cells alone may produce a very low amount of Ig without the help of accessory cells, the absolute B cell number was highly elevated. 1×10^6 /mL of B cells, approximately 10 times the B cell number in 1×10^6 /mL of PBMCs, produced mean $\pm$ SD 49.8 ± 5.6 ng/mL IgG, 35.0 ± 2.1 ng/mL IgM, and 10.0 ± 1.4 ng/mL IgA ($n = 6$). These amounts were still low but measurable. The same number of B cells treated with GQ1b (10 μ M/L) produced 49.7 ± 5.9 ng/mL IgG, 35.0 ± 2.6 ng/mL IgM, and 10.0 ± 2.1 ng/mL IgA, which were not significantly different from controls ($P > .8$, by Wilcoxon signed rank test). GQ1b did not affect the proliferation of B cells, either; ^{3} H-TdR uptake was mean $\pm$ SD 2823 ± 137 cpm in GQ1b-treated B cells compared with 2818 ± 161 cpm in control ($n = 6$). A colorimetric assay also showed similar results (data not shown). The results were similar when GQ1b concentration was variously changed from 0.01 to 100 μ M/L (data not shown). Thus GQ1b did not directly enhance Ig production of B cells, although it enhanced that of whole PBMCs. These findings indicate that GQ1b may act on accessory cells such as monocytes or T cells and enhance their function to help Ig production of B cells by releasing cytokines.

The inhibition by anti-cytokine antibodies of the GQ1b effects on PBMCs

To examine the involvement of cytokines in the stimulatory effects of GQ1b, antibodies against cytokines, which seemed relevant to Ig production, were added to the PBMC culture together with GQ1b, and their influ-

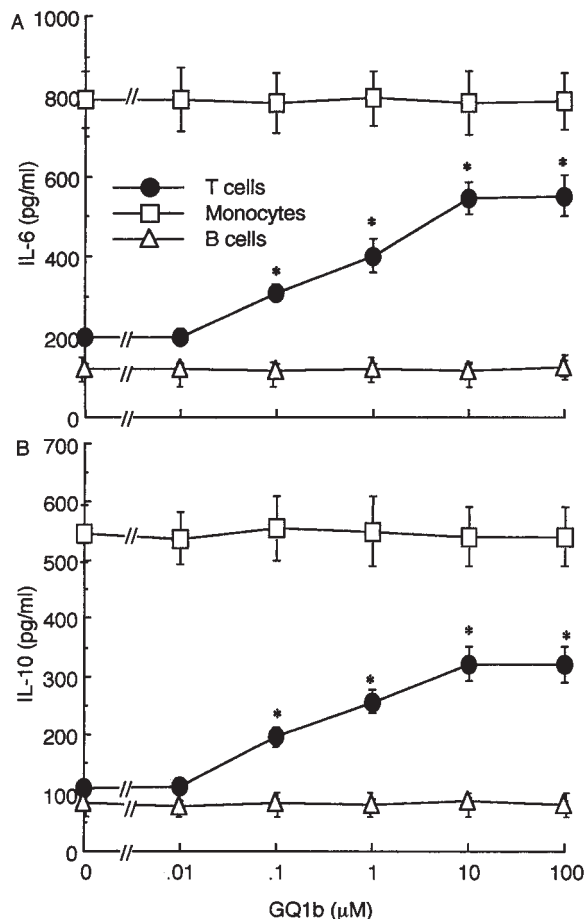


FIG 4. Dose-dependency of the GQ1b effects on IL-6 (**A**) and IL-10 (**B**) production. T cells, monocytes, and B cells (2×10^5 cells/200 mL/well) from 1 healthy donor were cultured in triplicate in the presence or absence of indicated doses of GQ1b for 24 hours, and the culture supernatants were analyzed for cytokines by ELISA. Bars show the mean $\pm$ SD of triplicate cultures. * $P < .05$ versus control cultures. The data represent 6 separate experiments using peripheral blood cells from 6 different donors.

ence was tested on the GQ1b effects. As shown in Fig 3, A, B, C, these antibodies were used each at 10 μ g/mL, which did not influence Ig production without GQ1b. GQ1b-induced enhancement of IgG, IgM, and IgA production was completely blocked by monoclonal anti-GQ1b antibody 7F5, indicating the specificity of the GQ1b effects. Among various anti-cytokine antibodies, anti-IL-6 and anti-IL-10 antibody each partially counteracted GQ1b-induced enhancement of IgG, IgM, and IgA production. When both antibodies were used together, additive inhibitory effects were revealed, and GQ1b-induced enhancement was completely blocked. These results suggest that IL-6 and IL-10 may be involved in the stimulatory effects of GQ1b on Ig production by PBMCs. The results also indicate that GQ1b may increase IL-6 and IL-10 production of PBMCs.

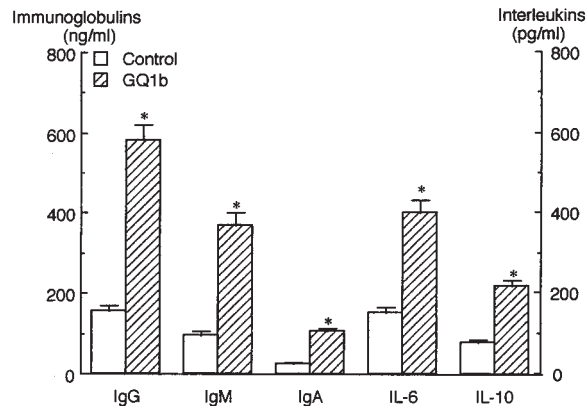


FIG 5. The effects of GQ1b on Ig and cytokine production in the culture of T and B cells. T cells (15×10^4 cells) and B cells (2×10^4 cells) from 1 healthy donor were cultured in the same well in triplicate with 200 μ L of medium or medium containing 10 μ mol/L of GQ1b. IgG, IgM, and IgA production were measured after 5 days, and IL-6 and IL-10 production were measured after 24 hours of culture by ELISA. Values are the mean $\pm$ SD of triplicate cultures. * $P < .05$ versus control cultures. The data represent 4 separate experiments using peripheral blood cells from 4 different donors.

The effects of GQ1b on cytokine production by PBMCs

To examine the effects of GQ1b on cytokine production by PBMCs, the culture supernatants with or without GQ1b were assayed for the activity of various cytokines. GQ1b significantly elevated IL-6 and IL-10 production of PBMC up to 206% and 211% of control, respectively (Table I). On the other hand, the production of IL-1 α , IL-1 β , IL-2, and IL-4 was not significantly changed by GQ1b. PBMCs were then subfractionated into T cells, monocytes, and B cells, and GQ1b effects on IL-6 and IL-10 production by each fraction were examined. As shown in Fig 4, A, B, IL-6 and IL-10 production in the absence of GQ1b are both highest in monocytes, followed by T cells and B cells. However, GQ1b did not affect IL-6 and IL-10 production of monocytes or B cells but only increased those of T cells; GQ1b at 10 μ mol/L elevated IL-6 and IL-10 production of T cells up to 277% and 295% of control, respectively. The dose-response curves for the GQ1b effects on IL-6 and IL-10 production were parallel to those for its effects on Ig production (Fig 1). Similarly to the entire PBMC culture, GQ1b did not affect the production of IL-1 α , IL-1 β , IL-2, and IL-4 by any fraction (data not shown). These results suggest that GQ1b may specifically increase IL-6 and IL-10 production of T cells. These results also indicate that such GQ1b effects on T cells may contribute to the enhancement of Ig production of B cells in whole PBMC culture. To confirm this contribution, we performed the following experiments. First, we examined whether GQ1b can increase Ig production of T-cell and B-cell culture without monocytes. As shown in Fig 5, GQ1b increased IgG, IgM, and IgA production of T-cell and B-cell culture, as well as that of IL-6 and IL-10. The increase of Ig

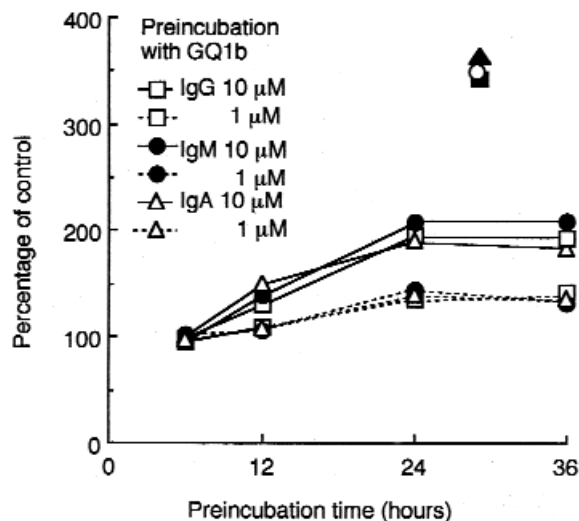


FIG 6. The effects of preincubation of T cells with GQ1b on Ig production. T cells (1×10^6 cells) from 1 healthy donor were preincubated with 1 mL of medium alone or medium containing 1 or 10 $\mu\text{mol/L}$ of GQ1b for indicated periods. After washing, T cells (15×10^4 cells) were added to B cells (2×10^4 cells) and monocytes (2×10^4 cells) from the same donor and cultured together with 200 μL of medium in triplicate for 5 days. IgG, IgM, and IgA production were measured as described in the legend for Fig 1. Results are expressed as the percentage versus control cultures preincubated with medium alone. In comparison, the same numbers of T cells, B cells, and monocytes were cultured together with GQ1b (10 $\mu\text{mol/L}$) for 5 days, and the percentages of IgG (■), IgM (●), and IgA (▲) amounts are shown versus control cultures with medium alone for the same period. The mean amounts of controls at 6, 12, 24, and 36 hours of preincubation were 317, 321, 341, and 331 ng/mL in IgG, 215, 227, 224, and 215 ng/mL in IgM, and 48, 51, 52, and 47 ng/mL in IgA, respectively. The mean amounts of controls of whole time incubation were 341 ng/mL IgG, 231 ng/mL IgM, and 58 ng/mL IgA. The data represent 3 separate experiments using peripheral blood cells from 3 different donors.

amounts may be attributed to GQ1b effects on T cells because Ig production of B cells alone was not increased by GQ1b. In the next experiment, we preincubated T cells with GQ1b, washed them, and then added them to B cells and monocytes, cultured together for 5 days, and examined whether Ig production may be enhanced by the pretreatment. As shown in Fig 6, the preincubation of T cells with GQ1b resulted in the enhancement of Ig production, although the effect was smaller than that of incubation of T cells, B cells, and monocytes together with GQ1b throughout the 5 days. The effect of preincubation is revealed at 12 hours, increased gradually, and maximized at 24 hours. Thus the results of these 2 experiments suggest that the stimulatory effects of GQ1b may be manifested by the GQ1b molecules incorporated into T cells.

Finally, we examined whether the addition of IL-6 and IL-10 may enhance Ig production of B cells alone. As shown in Table II, exogenous IL-6 and IL-10 each enhanced IgG, IgM, and IgA production of B cells, and the addition of both resulted in additive stimulatory effects on the Ig production.

IL-10 also enhanced ^3H -TdR uptake of B cells, whereas IL-6 did not have this effect. A colorimetric assay also showed similar results (data not shown). These results by exogenous cytokines suggest that endogenous IL-6 and IL-10, produced by accessory cells, may also increase Ig production of B cells in whole PBMC culture. Because GQ1b increased IL-6 and IL-10 production of T cells, GQ1b may indirectly enhance the Ig production of B cells by promoting the paracrine cytokine production of T cells in whole PBMCs.

DISCUSSION

In this study, gangliosides GQ1b, GM2, and GD1a enhanced and GT1b and GD1b inhibited spontaneous Ig production of human PBMCs. Among these gangliosides, the stimulatory effect of GQ1b was the most remarkable. GQ1b did not directly act on B cells but enhanced IL-6 and IL-10 production of T cells, and thus indirectly enhanced Ig production of B cells in whole PBMCs. Other gangliosides also enhanced or suppressed Ig production of B cells indirectly by modulating the function of accessory cells; details will be described in other papers (in preparation). The stimulatory effect of GD1a was mediated by promoting IL-6 and IL-10 production of monocytes, and that of GM2 was mediated by promoting IL-6 production of T cells. On the other hand, the inhibitory effect of GT1b was mediated by reducing IL-6 and IL-10 production of monocytes, and that of GD1b was mediated by reducing IL-6 and IL-10 production of T cells. Thus our study using PBMCs has revealed the effects of gangliosides on accessory cells, which was not mentioned in the previous studies. Our present results are different from those of the studies by Kimata and Yoshida.^{9,11} In their first study,⁹ GM2 and GM3 inhibited IgG and IgM production of *Staphylococcus aureus* Cowan strain I (SAC) plus IL-2-activated human tonsillar small resting B cells by reducing TNF- α production. Other gangliosides including GQ1b, GD1a, GT1b, and GD1b were ineffective. In their second study,¹¹ only GM2 inhibited IgG, IgM, and IgA production of human lymphoblastoid B-cell lines and in vivo activated B cells by reducing IL-10 and TNF- α production, whereas other gangliosides were ineffective. Several reasons are supposed for the difference between their results and ours; first, B cells or B cell lines were cultured alone in their study, and thus the effects of gangliosides on the accessory cells were not revealed. Second, they used in vivo or in vitro activated and differentiated B cells, whereas no B-cell stimuli were given in our study. Possibly GM2 and GM3 may only act on such activated B cells and may not act on resting B cells without stimuli. In our preliminary studies, GM2 and GM3 also inhibited Ig production of SAC plus IL-2-activated human peripheral blood small resting B cells by reducing TNF- α production. However, they were ineffective in the absence of SAC plus IL-2. Other gangliosides were ineffective either in the presence or absence of SAC and IL-2 (our unpublished observation).

Both IL-6 and IL-10 are known to differentiate B cells into Ig-secreting cells.²¹ In whole PBMC culture, these

cytokines are produced by monocytes, T cells, and also B cells themselves. These cytokines thus enhance Ig production of B cells through autocrine and paracrine mechanisms.²¹ GQ1b may not affect the autocrine loop but may amplify the paracrine mechanism via T cells. Although monocytes are the main source of IL-6 and IL-10 in PBMCs, GQ1b may not affect the production of these cytokines by monocytes (Fig 4). IL-10 also enhances the proliferation of B cells,²² which was also revealed in this study (Table II). However, the proliferation of whole PBMCs, including B cells, was not enhanced by GQ1b, although GQ1b elevated the IL-10 level of PBMCs (Table I). Some reasons are supposed for this result; first, the B-cell fraction is numerically small—approximately 10% of total PBMCs—and thus the IL-10-induced enhancement of B-cell proliferation may easily be obscured in whole PBMCs. Second, the concentration of IL-10 produced by GQ1b-treated PBMCs seems to be insufficient for growth-promoting effect; that was 200 to 400 pg/mL by 1×10^6 cells/mL of PBMCs (Table II). Previous studies also showed that less than 500 pg/mL of IL-10 did not enhance the proliferation of B cells.^{22,23}

How GQ1b may increase IL-6 and IL-10 production of T cells is not known. However, gangliosides, including GQ1b, interact with plasma membranes of various cells and transduce signals to the intracellular environment and thus control the cell growth and differentiation.²⁴⁻²⁶ GQ1b functions as such a signaling molecule in murine and human keratinocytes and induces the cornified envelope formation by generating 2 signals, protein kinase C activation and Ca^{2+} mobilization, from the intracellular stores.²⁷⁻²⁹ These 2 signals further trigger downstream signaling pathways, leading to the expression of the genes for type I transglutaminase, which synthesizes the cornified envelope.²⁸ These 2 signals, protein kinase C activation and intracellular Ca^{2+} mobilization, are also involved in the expression of IL-6 and IL-10 genes in T cells.³⁰⁻³² Therefore GQ1b may induce either or both of these signals in T cells in a manner similar to that in keratinocytes and thus enhance the expression of the genes for these cytokines. Although the stimulatory effects of GQ1b on IL-6 or IL-10 production by T cells have not been reported, GQ1b-coated liposomes increased the intracellular Ca^{2+} signals in rat T cells,³³ which indicates that the increased Ca^{2+} signals may induce IL-6 and/or IL-10 production in T cells. Also reported is that ganglioside GM1 enhanced IL-2 production in human T-cell lines via the increase of intracellular Ca^{2+} signals.^{25,34}

It is known that exogenous gangliosides are associated with cell membranes in different forms: (1) loose attachment by weak charge attractions and (2) stable association, either bound to membrane proteins or inserted into lipid bilayers.^{24,35} The biologic effects of gangliosides are generally attributed to the latter stable association.^{24,35} The stable association reportedly increases with increasing incubation time up to 24 hours.³⁶ This time-dependent association is compatible with our results, which show that the effect of T-cell preincubation with GQ1b is maximized at 24 hours (Fig 6). However, the

magnitude of the effect of preincubation is lower than that of whole-time incubation without washing, perhaps because membrane-associated gangliosides are internalized and catabolized in the lysosomes and thus lose their effects.²⁴ Why GQ1b specifically acts on T cells and not on B cells or monocytes is not known. No remarkable difference has been reported in GQ1b binding properties among T cells, B cells, and monocytes. Presumably, however, GQ1b may specifically interact with T-cell surface activation molecules such as CD3/T-cell receptor complexes, CD2, or CD4 molecules and thus trigger signaling pathways leading to IL-6 and/or IL-10 production.

Our results in vitro suggest that Ig production in vivo may also be regulated by gangliosides, which exist in the sera or tissue fluids of various organs.^{1,37} Because gangliosides are highly enriched in neuronal cells,¹ these cells may regulate Ig production locally in the central nervous system (CNS), as well as systemically. Although the CNS has long been considered a site of limited immune reactivity because of the blood-brain barrier, some studies suggest that humoral immunity is sustained in the CNS.³⁸⁻⁴⁰ GQ1b, GT1b, GD1b, and GD1a in particular are major gangliosides in the cerebrospinal fluid⁴¹ and thus may modulate intrathecal Ig synthesis. Among these gangliosides, stimulatory gangliosides GQ1b and GD1a may enhance the intrathecal Ig synthesis, whereas such stimulatory effects may be reversed by the inhibitory gangliosides GT1b and GD1b. These possibilities should further be investigated.

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