

Ganglioside GQ1b enhances anti-double-stranded DNA antibody and IgG production of PBMCs from patients with systemic lupus erythematosus

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Background: Previously, we reported that ganglioside GQ1b greatly enhanced spontaneous immunoglobulin production in vitro by PBMCs from normal human subjects.

Objective: We examined in vitro effects of GQ1b on anti-double-stranded DNA (anti-dsDNA) antibody production by PBMCs from patients with systemic lupus erythematosus (SLE).

Methods: PBMCs from patients with SLE were cultured with GQ1b. IgG anti-dsDNA antibody, total IgG, and cytokine amounts in the culture supernatants and protein kinase C (PKC) activity of T cells were measured by using ELISA. **Results:** GQ1b enhanced both anti-dsDNA and total IgG production of PBMCs from patients with SLE who were seropositive for anti-dsDNA. Among the seropositive patients, the active patients were more responsive to GQ1b in anti-dsDNA production than the inactive patients. GQ1b also enhanced total IgG production of PBMCs from patients with SLE who were seronegative for anti-dsDNA but did not induce their anti-dsDNA production. In contrast to PBMCs, GQ1b did not affect the antibody production either of purified CD5⁺ or of CD5⁻ B cells. Anti-IL-6 or anti-IL-10 antibody each partially blocked the GQ1b-induced enhancement of antibody production in PBMCs, and the addition of both antibodies completely blocked the enhancement. GQ1b increased IL-6 and IL-10 production of T cells. The supernatant from GQ1b-treated T cells enhanced antibody production both of CD5⁺ and of CD5⁻ B cells to a greater extent than that from medium-treated T cells. Exogenous IL-6 and IL-10 additively increased the antibody production both of CD5⁺ and CD5⁻ B cells. GQ1b-induced increases in IL-6 and IL-10 production of T cells were both blocked by PKC inhibitors, calphostin C and staurosporine. GQ1b enhanced PKC activity of T cells.

Conclusion: These results suggest that GQ1b may polyclonally increase the production of IgG, including IgG anti-dsDNA antibody, in PBMCs from patients with SLE by promoting IL-6 and IL-10 production of T cells through the enhancement of their PKC activity. (*J Allergy Clin Immunol* 2000;105:532-40.)

Key words: GQ1b, anti-double-stranded DNA antibody, IgG, systemic lupus erythematosus, IL-6, IL-10, T cell, protein kinase C

Abbreviations used

Anti-dsDNA:	Anti-double-stranded DNA
CNS:	Central nervous system
PKC:	Protein kinase C
SLE:	Systemic lupus erythematosus
SLEDAI:	Systemic lupus erythematosus disease activity index

Gangliosides are sialic acid-containing glycosphingolipids and are expressed on the plasma membranes of various cells.¹ Gangliosides are also extracellularly shed and exhibit various physiologic functions.¹ Several studies showed that gangliosides regulated human humoral immune responses in vitro.²⁻⁴ We also recently found that gangliosides GQ1b, GM2, and GD1a enhanced spontaneous IgG, IgM, and IgA production of PBMCs from normal human subjects in vitro, whereas this production was inhibited by GT1b and GD1b.⁵ Among these, the stimulatory effect of GQ1b was most remarkable, indicating that GQ1b in the sera or tissue fluids may also enhance humoral immunity in vivo. Because certain autoimmune diseases, such as systemic lupus erythematosus (SLE), are characterized by abnormally enhanced humoral immunity, it is hypothesized that GQ1b may be related to the development of these diseases. It is reported that B cells from patients with SLE spontaneously produce a large amount of immunoglobulins and autoantibodies in vitro.^{6,7} Among various autoantibodies, IgG anti-double-stranded DNA (anti-dsDNA) antibody is often associated with SLE and is related to the disease activity.⁸ It is thus anticipated that GQ1b may enhance IgG anti-dsDNA antibody production in patients with SLE. However, it has not yet been examined whether gangliosides modulate autoantibody production.

In this study we examined the in vitro effect of GQ1b on IgG anti-dsDNA antibody production in PBMCs from patients with SLE. The results showed the stimulatory effects of this ganglioside. Although the in vitro effects of GQ1b do not directly identify its in vivo function, they may be useful in a discussion of the possible role of GQ1b in the modulation of autoimmunity.

METHODS

Patients

We examined 20 patients with SLE (7 men and 13 women; age, 41.8 ± 12.2 years [mean ± SD]) whose sera were all positive for IgG

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anti-dsDNA antibody, as determined by using an ELISA kit (Fuji Rebio, Tokyo, Japan). Ten patients with SLE (3 men and 7 women; age, 41.6 ± 11.5 years [mean $\pm$ SD]) who were seronegative for IgG anti-dsDNA antibody were examined for purposes of comparison. All of the patients were informed of the objectives and methods of this study and provided consent to participate. All patients fulfilled the American College of Rheumatology criteria for SLE.⁹ Disease activity of the patients was scored on the basis of the SLE disease activity index (SLEDAI), as described previously.¹⁰ Twelve patients who had SLEDAI scores of 7 or less were considered to be inactive, and 18 patients with scores of 8 or more were considered to be active. Seven of the active patients presented the symptoms of central nervous system (CNS) lupus. Among 12 inactive patients, 4 were receiving no treatment, and 8 were receiving nonsteroidal anti-inflammatory drugs. Among 18 active patients, 12 were receiving 5 to 10 mg/d prednisolone, 4 were receiving 15 to 20 mg/d prednisolone, and 2 were receiving 40 mg/d prednisolone. The duration of the treatment with prednisolone ranged from 1 month to 10 years (4.84 ± 4.13 years).

Reagents

Highly purified bovine brain ganglioside GQ1b was 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 from Boehringer Mannheim (Indianapolis, Ind). Monoclonal mouse IgG anti-human IL-1 α and IL-1 β antibodies were from Immunotech (Westbrook, Me). Monoclonal mouse IgG anti-human IL-10 antibody and recombinant human IL-10 were from R&D Systems (Minneapolis, Minn). Monoclonal mouse IgG anti-GQ1b antibody 7F5 was from Mecto (Tokyo, Japan). Control mouse IgG was purchased from Dako (Tokyo, Japan). Staurosporine, calphostin C, herbimycin A, N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide (H-89), and calmidazolium chloride (R24571) were obtained from Calbiochem (La Jolla, Calif) and were dissolved in dimethylsulfoxide as 10 mmol/L stock solutions and kept in the dark until used.

Preparation of PBMCs, monocytes, T cells, and CD5⁺ or CD5⁻ B cells

Blood was drawn from patients with SLE, and PBMCs were isolated by centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). PBMCs were fractionated into monocytes, T cells, and B cells by the combination of plastic-dish adherence, sheep erythrocyte rosette formation, and immunomagnetic negative selection, as described.⁵ B cells were further divided into CD5⁺ and CD5⁻ fractions by panning with anti-CD5 antibody (Beckton Dickinson, Mountain View, Calif), as described.¹¹ The resultant CD5⁺ and CD5⁻ fractions contained greater than 90% CD5⁺ B cells and greater than 95% CD5⁻ B cells by flow cytometry, respectively.

Production of IgG anti-dsDNA antibody and total IgG

PBMCs and CD5⁺ or CD5⁻ B cells (2×10^5 /well) were cultured in triplicate in 96-well plates with or without GQ1b in 0.2 mL of culture medium at 37°C in 5% CO₂ for 5 days. To remove the influence of gangliosides in the serum, we used serum-free medium in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's Nutrient Mixture F-12 (Sigma) supplemented with 365.1 mg/L L-glutamine (Gibco/BRL, Grand Island, NY). This medium is hormone- and endotoxin-free and contains 2.0 mg/L hypoxanthine and 0.36 mg/L thymidine. GQ1b was dissolved in ethanol and diluted at least 1000-fold to the required concentration in the medium. Control cultures contained ethanol at 0.1%, which was not toxic to PBMCs. The culture supernatants were harvested. Total IgG and

IgG anti-dsDNA antibody amounts in the supernatants were measured by ELISA, as described.^{12,13} Samples containing 5.0 U/mL or greater anti-dsDNA antibody were considered positive, as described.¹³ No cross-reactivity to our anti-dsDNA assay was shown by the sera positive for IgG anti-single-stranded DNA antibody but negative for IgG anti-dsDNA antibody in commercially available ELISA kits (Fuji Rebio).

Production of cytokines

PBMCs or their subpopulations (2×10^5 /well) were cultured in triplicate in 96-well plates for 2 days in 0.2 mL of medium with or without GQ1b. The cytokine activity in the culture supernatants was measured by using ELISA kits (Endogen, Cambridge, Mass). The detection limits 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.

Protein kinase C assays

T cells (1×10^6 /mL) were cultured in the medium for 1 hour with or without GQ1b (10 μ mol/L), washed, and assayed for protein kinase C (PKC) activity by using ELISA kits (Medical and biological laboratories, Nagoya, Japan) according to the manufacturer's protocol.

Statistical analysis

Data analysis was performed with the 1-way ANOVA with Dunnett's multiple comparison test, the paired *t* test, and the 1-way ANOVA with Scheffe's multiple comparison test. A *P* value of less than .05 was considered significant.

RESULTS

Stimulatory effects of GQ1b on antibody production by PBMCs

First, we added various concentrations of GQ1b to PBMCs from patients with SLE who were seropositive for IgG anti-dsDNA antibody. An active patient's PBMCs constitutively produced more than the threshold (5 U/mL) of anti-dsDNA, and GQ1b enhanced both anti-dsDNA antibody and total IgG production of the PBMCs to a similar extent (Fig 1). The stimulatory effect of GQ1b was detected at 0.1 μ mol/L, increased in a dose-dependent manner, and was maximized at 10 μ mol/L. An inactive patient's PBMCs constitutively produced a very low amount of anti-dsDNA (<5 U/mL), and the antibody production in the presence of GQ1b appeared slightly higher, although it was still below the threshold; the difference from control was not significant. Total IgG production of the inactive patient's PBMCs was enhanced by GQ1b to the extent similar to that found in the active patient. Ten micromoles per liter seemed to be the optimal concentration for the GQ1b effects and was thus used in subsequent experiments.

In the next experiment GQ1b (10 μ mol/L) was added to PBMCs from 20 patients who were seropositive for anti-dsDNA. The effect of GQ1b was examined in the active and inactive groups, respectively. The control anti-dsDNA amounts increased with increasing SLEDAI scores ($r_s = 0.847$, $P < .001$), whereas those of total IgG did not correlate with the scores ($r_s = 0.131$, $P > .5$). As shown in Fig 2, A, 6 (50%) of 12 active patients constitutively produced 5 U/mL or greater anti-dsDNA, where-

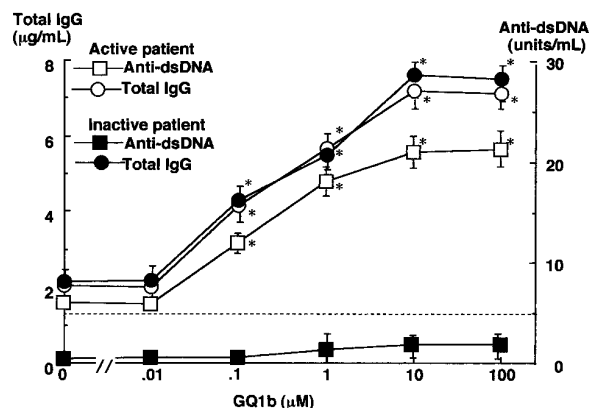


FIG 1. Dose dependency for the effect of GQ1b on the antibody production by PBMCs from patients with SLE who were seropositive for anti-dsDNA antibody. PBMCs from an active or inactive patient were cultured with GQ1b for 5 days and were assayed for IgG anti-dsDNA antibody and total IgG production. Values are the means $\pm$ SD of triplicate cultures. The dashed line indicates the cutoff point for the presence or absence of anti-dsDNA. Data represent 6 separate experiments with PBMCs from 3 active patients and 3 inactive patients. Analysis of data was performed with 1-way ANOVA with Dunnett's multiple comparison test (* $P < .01$ versus control levels).

as none of the 8 inactive patients did so. The anti-dsDNA amount was increased by GQ1b up to 3.62-fold of control on average, and the difference was significant both in the active ($P < .01$) and inactive ($P < .05$) groups.

GQ1b also elevated the total incidence of anti-dsDNA (ie, the percentage of patients with 5 U/mL or greater) from 30% (6/20) to 70% (14/20), and the difference was significant ($P < .05$, as determined by using Fisher's exact test). In the inactive group, however, the mean antibody amount in the presence of GQ1b was still less than 5 U/mL. The incidence of this antibody in the presence of GQ1b was only 25% (2/8) in the inactive group, whereas it was 100% (12/12) in the active group. These suggest the lower responsiveness to GQ1b in the inactive group.

In the active group the anti-dsDNA amount in the presence of GQ1b correlated with the control antibody amount ($r_s = 0.938$, $P < .01$) and with SLEDAI score ($r_s = 0.693$, $P < .05$); 6 patients constitutively producing 5 U/mL or greater (35.2 ± 10.0 U/mL [mean $\pm$ SD]) could produce a further increased amount in response to GQ1b (127.7 ± 37.6 U/mL; upper 6 points in the leftmost panel of Fig 2, A), whereas the other 6 patients constitutively producing less than 5 U/mL (4.2 ± 0.4 U/mL) produced 14.9 ± 1.7 U/mL in response to GQ1b, which was increased but still lower than the antibody amount of the former subgroup ($P < .01$, as determined by using the Mann-Whitney U test). The patients of the former subgroup had higher SLEDAI scores (15.66 ± 1.50) and were receiving higher doses (range, 15–40 mg/d; mean $\pm$ SD, 24.6 ± 12.1 mg/d) of prednisolone compared with the latter subgroup having SLEDAI scores of 9.34 ± 1.64 and lower prednisolone doses (range, 5–10 mg/d; mean $\pm$ SD, 6.7 ± 2.0 mg/d; $P < .01$ for both comparisons, as determined by using the Mann-Whitney U test). We then analyzed the relationship

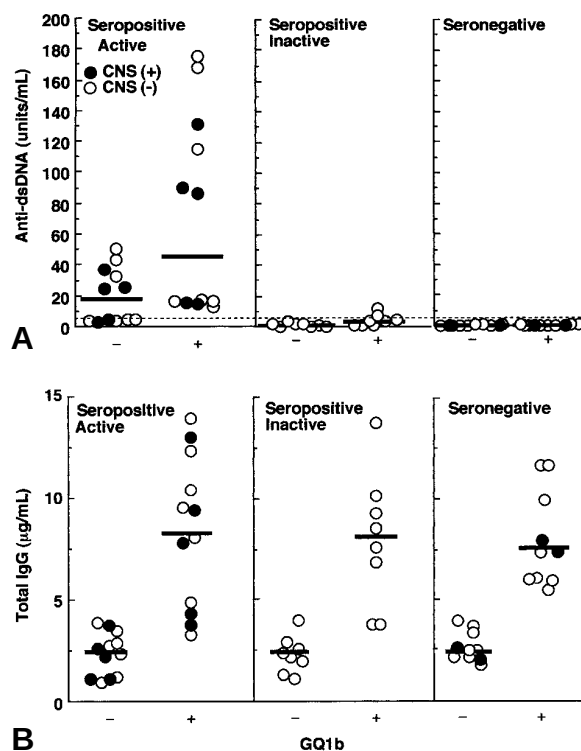


FIG 2. Stimulatory effect of GQ1b on antibody production by PBMCs from 12 active and 8 inactive patients with SLE who were seropositive for anti-dsDNA and 6 active and 4 inactive patients with SLE who were seronegative for this antibody. PBMCs were cultured in the presence (+) or absence (-) of GQ1b (10 μ mol/L) for 5 days and were assayed for anti-dsDNA (A) and total IgG (B) production. Each point represents the mean amount of triplicate cultures from individual donors. The solid lines show the group means. The dashed lines indicate the cutoff point for the presence or absence of anti-dsDNA. Analysis of data was performed with the paired t test.

between the anti-dsDNA production and the duration of prednisolone use. Six active patients treated with prednisolone for 4 or more years produced 18.7 ± 18.4 U/mL constitutively and 66.3 ± 64.4 U/mL in response to GQ1b. The other 6 active patients treated for less than 4 years produced 20.7 ± 18.3 U/mL constitutively and 76.3 ± 69.7 U/mL in response to GQ1b. There was no significant difference in the antibody amounts of the 2 subgroups, either in the presence or absence of GQ1b ($P > .8$ for both comparisons, as determined by the Mann-Whitney U test). We also analyzed the relationship between the anti-dsDNA production and CNS lupus. Five active patients with CNS lupus produced 22.7 ± 13.3 U/mL constitutively and 67.7 ± 51.1 U/mL in response to GQ1b. The 7 active patients without CNS lupus produced 20.2 ± 18.7 U/mL constitutively and 73.9 ± 66.1 U/mL in response to GQ1b. There was no significant difference in the antibody amounts of the 2 subgroups, either in the presence or absence of GQ1b ($P > .8$ for both comparisons, as determined by using the Mann-Whitney U test).

The mean total IgG amount was increased by GQ1b up to 3.55-fold of control on average, and the difference was significant both in the active ($P < .01$) and inactive (P

TABLE I. The effects of GQ1b on the antibody production of CD5⁺ or CD5⁻ B cells

Conditions	Anti-dsDNA (U/mL)		Total IgG (μg/mL)	
	CD5 ⁺ B cells	CD5 ⁻ B cells	CD5 ⁺ B cells	CD5 ⁻ B cells
Control	12.6 ± 0.8	10.5 ± 1.0	1.52 ± 0.29	1.49 ± 0.31
GQ1b	12.3 ± 0.9	10.6 ± 0.8	1.51 ± 0.30	1.49 ± 0.28

CD5⁺ or CD5⁻ B cells from active patients with SLE who were seropositive for anti-dsDNA were cultured with or without GQ1b (10 μmol/L). The antibody production was evaluated after 5 days. Values are means ± SD (n = 6; 3 with CNS lupus and 3 without). Analysis of data was performed with the paired *t* test.

< .05) groups (Fig 2, B). There was no significant difference in the total IgG amounts of the 2 groups, either in the presence or absence of GQ1b (*P* > .8 for both comparisons, as determined by using the Mann-Whitney *U* test). Thus in total IgG production, there seemed no difference between the 2 groups in the responsiveness to GQ1b.

In comparison with the results in patients who were seropositive for anti-dsDNA, the effect of GQ1b was examined in 10 patients who were seronegative for this antibody. Either in the presence or absence of GQ1b, these patients' PBMCs did not produce 5.0 U/mL or greater anti-dsDNA. Thus GQ1b did not induce anti-dsDNA production in seronegative patients. In contrast, the total IgG amount in these patients was increased by GQ1b up to 3.18-fold of control on average, and the difference was significant (*P* < .01).

Direct effect of GQ1b on antibody production by CD5⁺ or CD5⁻ B cells

Because GQ1b effect on anti-dsDNA production was remarkable in the 6 patients constitutively producing 5.0 U/mL or greater in vitro (upper 6 points in the leftmost panel of Fig 2, A), the following experiments were performed on these patients. We examined the direct effect of GQ1b on B cells of these patients. Because some previous studies^{14,15} suggest that CD5⁺ B cells may be an autoantibody-producing subset, B cells were divided into CD5⁺ and CD5⁻ fractions, and the direct GQ1b effects on both fractions were examined. Both fractions constitutively produced anti-dsDNA and total IgG (Table I). GQ1b did not directly enhance the antibody production either of CD5⁺ or of CD5⁻ B cells. It is thus indicated that GQ1b may act on accessory cells like monocytes or T cells and augment their function to help B-cell antibody production by means of releasing cytokines.

Inhibition of the GQ1b effect by anticytokine antibodies

To examine the involvement of cytokines in the GQ1b effect, various anticytokine antibodies were added to PBMCs cultured with GQ1b, and their influence on the GQ1b effect was tested. GQ1b-induced enhancement of antibody production was completely blocked by anti-GQ1b antibody, indicating the specificity of the GQ1b effect (Fig 3). Among various anticytokine antibodies, anti-IL-6 or anti-IL-10 antibody each partially blocked GQ1b-induced enhancement of both anti-dsDNA and total IgG production. When both antibodies were used together, additive inhibitory effects were detected, and

GQ1b-induced enhancement was completely blocked. These results suggest that IL-6 and IL-10 may be involved in the GQ1b effect, indicating that GQ1b may increase IL-6 and IL-10 production of PBMCs.

Effect of GQ1b on cytokine production

We then examined the effects of GQ1b on cytokine production by PBMCs. GQ1b increased IL-6 and IL-10 production by PBMCs up to 2.32-fold and 2.03-fold of control on average, respectively (Table II). In contrast, the production of the other cytokines was not altered by GQ1b. PBMCs were then fractionated into monocytes, T cells, and CD5⁺ or CD5⁻ B cells, and GQ1b effects on IL-6 and IL-10 production by each fraction were examined. GQ1b only increased IL-6 and IL-10 production of T cells and did not affect those of other PBMC subpopulations (Fig 4). GQ1b (10 μmol/L) elevated IL-6 and IL-10 production of T cells up to 2.32-fold and 2.19-fold of control levels, respectively. GQ1b did not affect the production of IL-1α, IL-1β, IL-2, and IL-4 by any fraction (data not shown). Thus GQ1b specifically increased both IL-6 and IL-10 production of T cells. It is indicated that the GQ1b effects on T cells may contribute to the enhancement of B cell antibody production.

To confirm this contribution, the following experiments were performed. First, we examined whether the supernatant from GQ1b-treated T cells may enhance B cell antibody production. IL-6 and IL-10 amounts in the GQ1b-treated T-cell supernatant were 2.68-fold and 2.51-fold of the amounts in the medium-treated T-cell supernatant, respectively (Fig 5, A). Both of these supernatants enhanced anti-dsDNA and total IgG production of CD5⁺ and CD5⁻ B cells (Fig 5, B). However, the effect of GQ1b-treated T-cell supernatant was much greater than that of the medium-treated supernatant. GQ1b did not directly enhance either CD5⁺ or CD5⁻ B cell antibody production. Thus the B-cell stimulatory effect of the GQ1b-treated T cell supernatant may not be the direct action of the included GQ1b molecules but may be attributable to GQ1b-induced cytokines, possibly IL-6 and IL-10.

In the next experiment we examined whether the addition of IL-6 and IL-10 may enhance the antibody production of CD5⁺ or CD5⁻ B cells. Exogenous IL-6 or IL-10 individually enhanced anti-dsDNA and total IgG production of both B-cell fractions (Table III). The addition of both cytokines resulted in additive stimulatory effects on the antibody production. These results by exogenous cytokines suggest that endogenous IL-6 and IL-10 produced by T cells may also increase the B-cell

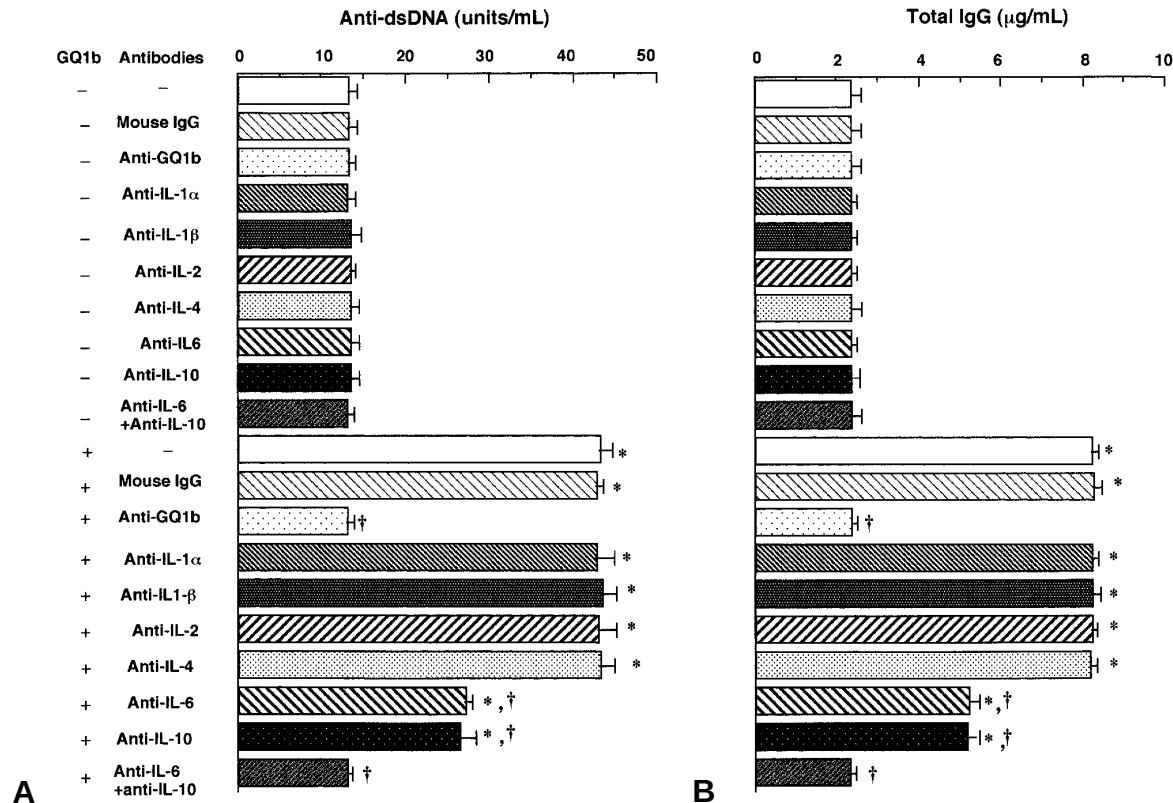


FIG 3. Inhibition of the GQ1b effect by various antibodies. PBMCs from active patients with SLE who were seropositive for anti-dsDNA were cultured with (+) or without (-) GQ1b (10 μ mol/L) in the presence or absence of various antibodies (each 10 μ g/mL) for 5 days and were assayed for anti-dsDNA (**A**) and total IgG (**B**) production. The concentration of antibodies was determined as recommended by the manufacturers. Values are means $\pm$ SD ($n = 6$; 3 with CNS lupus and 3 without). * $P < .001$ versus controls with medium alone. † $P < .001$ versus cultures with GQ1b alone. Analysis of data was performed with 1-way ANOVA with Scheffe's multiple comparison test.

TABLE II. 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	54 $\pm$ 25	26 $\pm$ 13	36 $\pm$ 23	21 $\pm$ 12	339 $\pm$ 54	222 $\pm$ 39
GQ1b	54 $\pm$ 23	25 $\pm$ 15	37 $\pm$ 21	21 $\pm$ 11	681 $\pm$ 77*	450 $\pm$ 76*

PBMCs from active patients with SLE who were seropositive for anti-dsDNA were cultured for 2 days with or without GQ1b (10 μ mol/L) and were assayed for cytokine production. Values are means $\pm$ SD ($n = 6$; 3 with CNS lupus and 3 without). Analysis of data was performed with the paired t test.

* $P < .05$ versus controls.

TABLE III. The effects of IL-6 and IL-10 on antibody production of CD5⁺ or CD5⁻ B cells

Conditions	Anti-dsDNA (U/mL)		Total IgG (μ g/mL)	
	CD5 ⁺ B cells	CD5 ⁻ B cells	CD5 ⁺ B cells	CD5 ⁻ B cells
Control	13 $\pm$ 1	12 $\pm$ 1	1.4 $\pm$ 0.3	1.6 $\pm$ 0.3
IL-6	27 $\pm$ 2 (116)*	27 $\pm$ 2 (125)*	3.3 $\pm$ 0.4 (112)*	3.5 $\pm$ 0.6 (119)*
IL-10	30 $\pm$ 3 (134)*	29 $\pm$ 2 (143)*	3.6 $\pm$ 0.3 (133)*	3.9 $\pm$ 0.5 (144)*
IL-6 plus IL-10	44 $\pm$ 3 (249)*†‡	44 $\pm$ 4 (269)*†‡	5.4 $\pm$ 0.4 (246)*†‡	5.9 $\pm$ 0.7 (269)*†‡

CD5⁺ or CD5⁻ cells from active patients with SLE who were seropositive for anti-dsDNA were cultured with or without IL-6, IL-10, or both (each 600 pg/mL). The antibody production was evaluated after 5 days. Values are means $\pm$ SD ($n = 6$; 3 with CNS lupus and 3 without), and values in parentheses are percentage increase versus control levels. Analysis of data was performed with the 1-way ANOVA with Scheffe's multiple comparison test.

* $P < .01$ versus controls.

† $P < .01$ versus cultures with IL-6 alone.

‡ $P < .01$ versus cultures with IL-10 alone.

antibody production. Thus GQ1b may indirectly enhance B-cell antibody production in whole PBMCs by promoting the paracrine IL-6 and IL-10 production of T cells.

Signaling pathways involved in GQ1b effects on cytokine production

It is known that gangliosides modulate a variety of transmembrane signaling pathways, especially those regulating the activity of protein kinases.¹⁶ Thus we tried to elucidate the signaling pathway involved in GQ1b-induced enhancement of IL-6 and IL-10 production by T cells. We preincubated T cells with several signal transduction inhibitors and then stimulated them with GQ1b and examined the influence of these inhibitors on GQ1b effects on the cytokine production. PKC inhibitors, staurosporine, and calphostin C completely inhibited the GQ1b-induced enhancement of both IL-6 and IL-10 production (Fig 6). In contrast, protein tyrosine kinase inhibitor herbimycin A, protein kinase A inhibitor H-89, and calmodulin antagonist R24571 did not suppress the GQ1b effects. These results suggest the involvement of PKC in the GQ1b effects on the cytokine production, indicating the enhancement of PKC activity by GQ1b. We thus compared the PKC activity of GQ1b-treated T cells and that of controls treated with medium alone. The activity of PKC, as expressed as an optical density at 492 nm, was enhanced by GQ1b (1.523 ± 0.112 in GQ1b-treated T cells compared with 0.852 ± 0.073 in controls; $n = 6$; $P < .05$, as determined by using a paired *t* test). These results suggest that GQ1b may promote PKC activity in T cells, and this may lead to the increased production of IL-6 and IL-10.

DISCUSSION

In this study GQ1b enhanced the anti-dsDNA production of PBMCs from patients with SLE in vitro. Fig 7 shows the putative immunologic pathways activated by GQ1b. GQ1b may promote PKC activity of T cells and trigger the downstream signals leading to IL-6 and IL-10 production, thus enhancing the secretion of these cytokines. The increase of IL-6 and IL-10 may facilitate the differentiation of B cells and thus enhance the antibody secretion. Recently, we have also found that GQ1b in vitro enhances anticardiolipin antibody production of PBMCs from patients with SLE and also enhances anti-SS-A antibody production of PBMCs from patients with Sjogren's syndrome (unpublished observations). It appears that GQ1b may polyclonally increase IgG production, and its effect may not be specific for IgG anti-dsDNA antibody because the enhancement of this antibody production paralleled that of total IgG. In our previous study GQ1b also enhanced total IgG production in normal donors' PBMCs by increasing IL-6 and IL-10 production of their T cells.⁵

GQ1b did not induce anti-dsDNA production in patients with SLE who were seronegative for this antibody. Among seropositive patients, 6 active patients constitutively producing anti-dsDNA in vitro (Fig 2, A) were

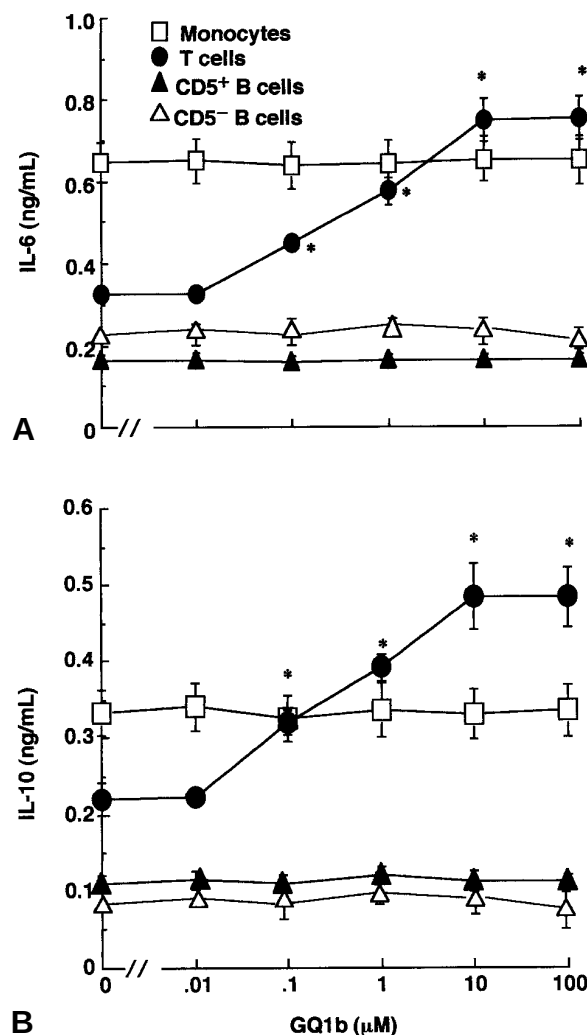


FIG 4. Dose dependency of the GQ1b effect on IL-6 (A) and IL-10 (B) production. T cells, monocytes, and CD5⁺ or CD5⁻ B cells from an active patient with SLE who was seropositive for anti-dsDNA were cultured with indicated doses of GQ1b for 2 days and were assayed for cytokine production. Values are the means $\pm$ SD of triplicate cultures. Analysis of data was performed with 1-way ANOVA with Dunnet's multiple comparison test (* $P < .05$ versus control levels). Data represent 6 separate experiments with peripheral blood cells from 6 active patients with SLE who were seropositive for anti-dsDNA (3 with CNS lupus and 3 without).

much more responsive to GQ1b in this antibody production than the others. These results may reflect the difference in the number of anti-dsDNA-producing B cells. Possibly in the 6 active patients above, considerable numbers of B cells may be induced in vivo to produce this antibody. Such induction may be due to B-cell hyperactivation by polyclonal activators (eg, viruses),⁷ clonally selective stimulation by self antigens (eg, nucleosomes),¹⁷ or both.¹⁸ In contrast, such in vivo induction may be weak or may not occur in the other patients, and thus they may have no or few anti-dsDNA-producing subsets. GQ1b may further enhance the anti-dsDNA production of those subsets indirectly through T cells; however, it may not turn non-anti-dsDNA-producing B cells

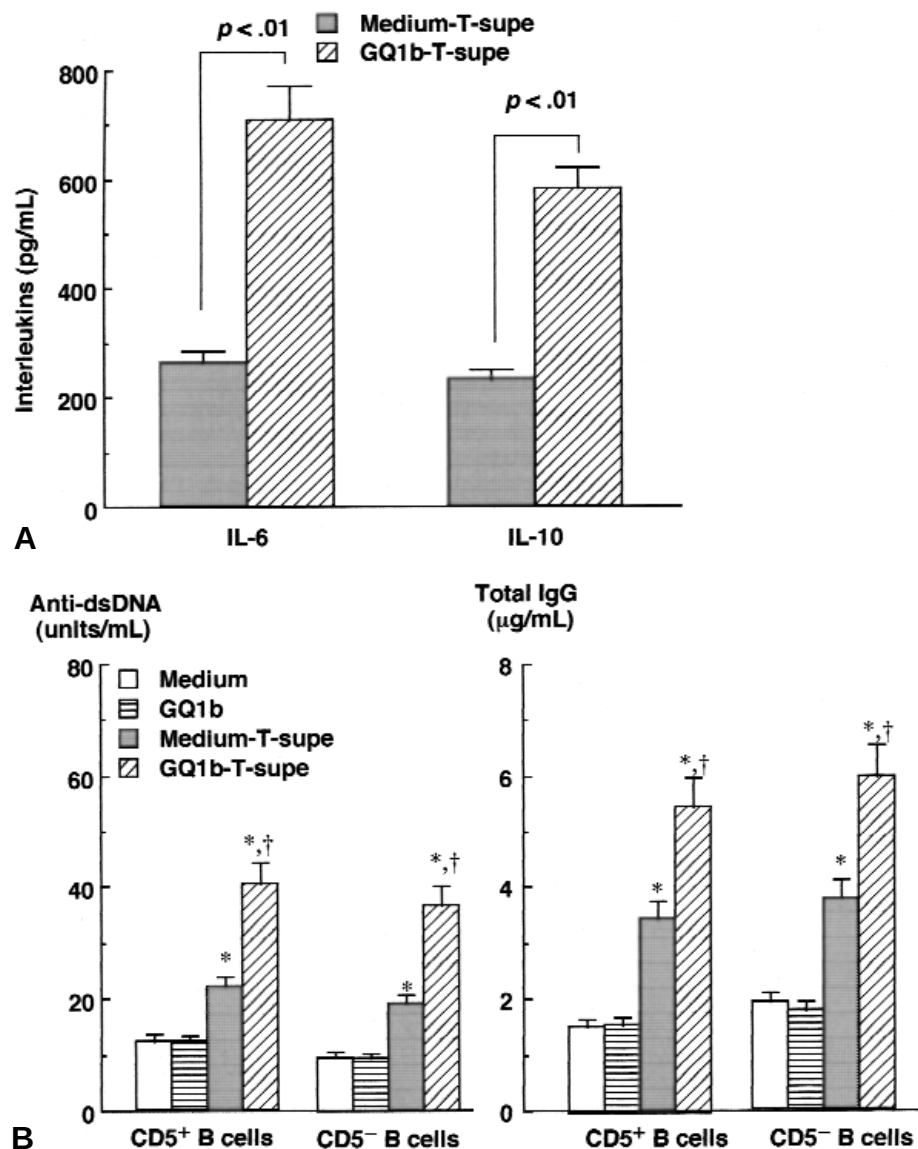


FIG 5. IL-6 and IL-10 amounts in the supernatant from GQ1b- or medium-treated T cells (**A**) and the effects of the supernatants on B-cell antibody production (**B**). T cells (1×10^6 cells/mL) from 6 active patients with SLE who were seropositive for anti-dsDNA were cultured with or without GQ1b ($10 \mu\text{mol/L}$) for 2 days. The culture supernatants were harvested, and 200- μL aliquots were assayed for IL-6 and IL-10. One hundred fifty-microliter aliquots of the supernatants were added to CD5⁺ or CD5⁻ B cells (2×10^5 cells in 50 μL of fresh medium) from the identical donors, and the B cell fractions were cultured in triplicate. The identical B-cell fractions were simultaneously cultured with medium alone or medium containing GQ1b ($7.5 \mu\text{mol/L}$). The B-cell antibody production was evaluated after 5 days. Values are means $\pm$ SD ($n = 6$; 3 with CNS lupus and 3 without). Analysis of data in **A** was performed with the paired t test, and analysis of data in **B** was performed with the 1-way ANOVA with Scheffe's multiple comparison test (* $P < .05$ versus control cultures with medium alone; † $P < .05$ versus cultures with the supernatant from medium-treated T cells).

into producers. Although some studies support the hypothesis that human CD5⁺ B cells may be an anti-dsDNA-producing subset,^{14,15} others deny the possibility.^{19,20} At least in the present study, the presence of anti-dsDNA-producing cells may not be limited to CD5⁺ B cell fraction because both CD5⁺ and CD5⁻ B cell fractions constitutively produced anti-dsDNA in vitro.

GQ1b increased IL-6 and IL-10 production of T cells

by promoting their PKC activity. It is known that PKC activation is required for IL-6 and IL-10 gene expression in T cells.^{21,22} It is hypothesized that the binding of GQ1b to the T-cell plasma membrane may produce a conformational change that leads to an increase in the affinity of PKC for cofactors. Alternatively, the GQ1b binding may promote the translocation of PKC from cytosol to membrane, which is reported in mouse kera-

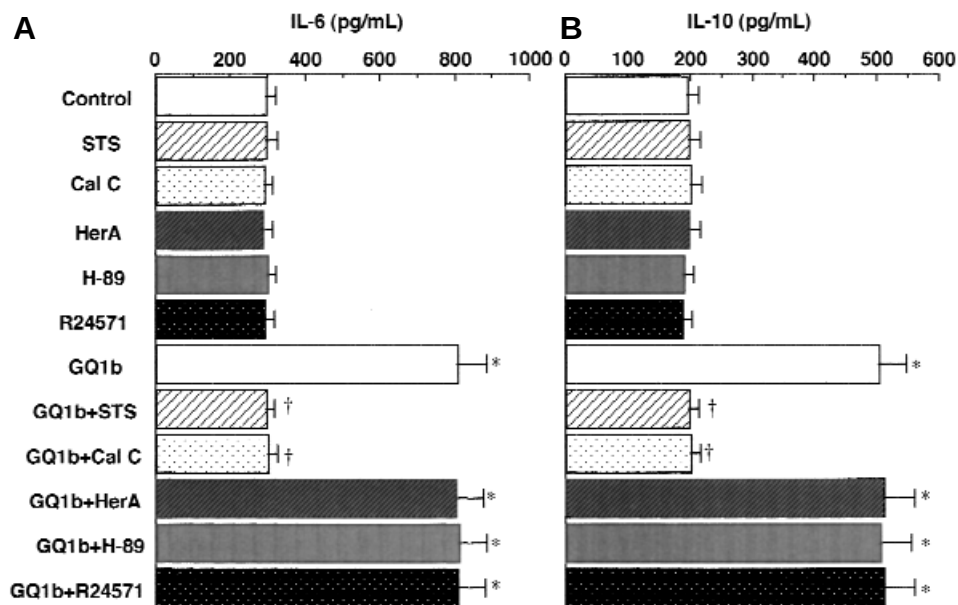


FIG 6. The effects of signal transduction inhibitors on GQ1b-induced IL-6 and IL-10 production. T cells ($2 \times 10^5/200 \mu\text{L}/\text{well}$) from 6 active patients with SLE who were seropositive for anti-dsDNA were preincubated for 1 hour with medium alone or with medium containing signal transduction inhibitors before the addition of GQ1b ($10 \mu\text{mol/L}$). After 2 days, the culture supernatants were assayed for IL-6 (**A**) and IL-10 (**B**). The inhibitors were staurosporine (STS; final 1.5 nmol/L), calphostin C (Cal C; $0.1 \mu\text{mol/L}$), herbimycin A (HerA, $1 \mu\text{mol/L}$), and calbidazolium chloride (R24571, $1 \mu\text{mol/L}$). The concentration of each inhibitor was determined to inhibit the aimed signal specifically as recommended by the manufacturer. Values are means $\pm$ SD ($n = 6$; 3 with CNS lupus and 3 without). Analysis of data was performed with the 1-way ANOVA with Scheffe's multiple comparison test (* $P < .001$ versus control cultures with medium alone; † $P < .001$ versus cultures with GQ1b alone).

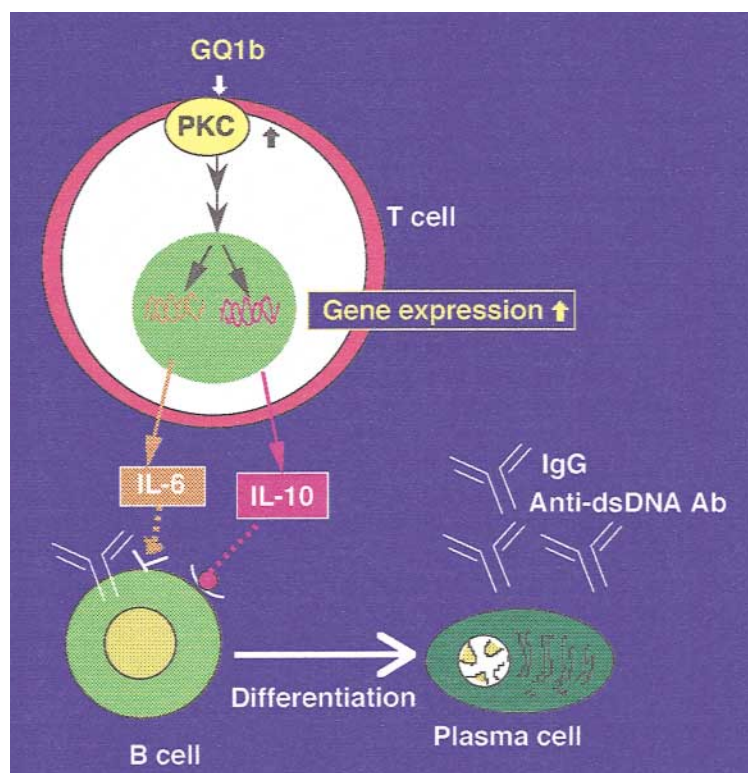


FIG 7. A schematic diagram for putative immunologic pathways activated by GQ1b. Ab, Antibody.

tinocytes.²³ On the T-cell membrane, GQ1b may bind to certain activation molecules linked to PKC, such as CD3/T cell receptor complex or CD4 molecules, and such binding may trigger the activation of PKC. The putative GQ1b target molecules may be specific to T cells because GQ1b did not alter IL-6 or IL-10 production of the other PBMC subpopulations.

Our results obtained *in vitro* indicate that GQ1b may also stimulate antibody production in patients with SLE *in vivo*. Because human serum GQ1b level is low (<0.2 $\mu\text{mol/L}$)²⁴ and its increase in patients with SLE is not reported, the contribution of GQ1b to systemic antibody production may be weak. However, GQ1b is enriched in the brain, and its level in the human occipital lobe is 14.8 $\mu\text{mol/kg}$ wet tissue weight.²⁵ This indicates that T cells in the CNS may be at least locally exposed to GQ1b at high concentrations, which would enhance IL-6 and IL-10 production, and that GQ1b may stimulate intrathecal antibody production. In the present *in vitro* study, GQ1b did stimulate PBMCs from patients with CNS lupus, although it was also stimulatory for the patients without CNS lupus. Several studies reported that the cerebrospinal fluids of patients with CNS lupus showed the presence of autoantibodies, including anti-dsDNA, and an increased IgG index, an indicator of intrathecal IgG production.²⁶ These suggest activated intrathecal antibody production in these patients. It is reported that the IL-6 level in cerebrospinal fluid is increased in the patients with CNS lupus.²⁷ Collectively, it is hypothesized that GQ1b may enhance the intrathecal antibody production of B cells by promoting IL-6 production of T cells in these patients. On the other hand, the CNS also contains high levels of GT1b and GD1b,²⁵ which suppressed *in vitro* immunoglobulin production of normal human PBMCs.⁵ These gangliosides may also inhibit the antibody production in patients with SLE and reverse the stimulatory effect of GQ1b. This possibility is now under investigation.

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