Differential Effects of Gangliosides on Ig Production and Proliferation by Human B Cells

By H. Kimata and A. Yoshida

The effects of gangliosides on human B-cell responses were studied. Of various gangliosides tested, only GM2 and GM3 inhibited production of IgG subclasses and IgM, but not IgA subclasses, and thymidine uptake by human B cells stimulated with SAC plus interleukin-2 (IL-2). In contrast, GM1, GD1a, GD1b, GD3, GT1b, and GG1b were without effects. GM2- and GM3-induced inhibition were specific, because each was blocked by a corresponding antibody. Of various cytokines tested, tumor necrosis factor-α (TNF-α) alone counteracted GM2- and GM3-induced inhibitions of Ig production and thymidine uptake, whereas other cytokines including IL-1β, IL-3, IL-5, IL-6, and interferon-γ each failed to do so. Moreover, anti-TNF-α antibody, but not control IgG, blocked the counteraction of inhibition by TNF-α. GM2 and GM3 each inhibited Ig production, thymidine uptake, and TNF-α production by surface IgG (slgG1*), slgG2*, slgG3*, slgG4*, and slgM* B cells without affecting IL-2 binding or TNF-α binding to B cells, but had no such inhibitory effects on slgA1* or slgA2* B cells. These findings indicate that GM2 and GM3 inhibit Ig production and thymidine uptake by human slgG1*, slgG2*, slgG3*, slgG4*, and slgM* B cells by inhibiting endogenous TNF-α production.

© 1994 by The American Society of Hematology.
(0.01%), IL-2 (300 U/mL), and IL-6 (100 U/mL), and incubated with the gangliosides for 7 days. Production of Ig (IgG1, IgG2, IgG3, IgG4, IgM, IgA1, and IgA2) was measured by enzyme-linked immunosorbent assay (ELISA).\(^1\)\(^2\) For measurement of proliferation, the B cells were cultured as above for 4 days (unless otherwise indicated), pulsed with 1 \(\mu\)Ci \(^3\)H-thymidine, and harvested after 16 hours. Thymidine uptake was then determined.\(^1\)\(^2\) The sensitivity of the assay for each Ig measurement was 0.3 ng/mL.\(^1\)\(^2\) In another experiment, cells were cultured for 3 days and TNF-\(\alpha\) production was determined in culture supernatants by ELISA (R & D Systems, Minneapolis, MN). The lower limit of the detection of the assay was 15 pg/mL. Cultured cells were also tested for binding of IL-2 and TNF-\(\alpha\) by immunofluorescence using biotinylated IL-2 and TNF-\(\alpha\) as previously reported.\(^2\)\(^4\) Briefly, B cells were cultured for 2 days and were then washed with acidic buffer to remove surface-bound IL-2 or TNF-\(\alpha\). Cells were incubated for 3 hours at 4\(^\circ\)C in the presence of 5 nmol/L of biotinylated IL-2 or TNF-\(\alpha\), and then stained with streptavidin-phycoerythrin (PE) and analyzed with a FACScan. The mean fluorescence intensity value of biotinylated ligand-specific binding was determined after subtraction of the nonspecific binding in the presence of a 100-fold excess of unlabeled ligand and expressed as \(\Delta MF\).\(^2\)\(^4\) Statistical analysis was performed with the two-tailed Student’s \(t\)-test.

### RESULTS

**Effect of gangliosides on Ig production and thymidine uptake by B cells.** As shown in Fig 1A, GM2 and GM3 inhibited IgG1 production in a dose-dependent fashion, whereas GM1, GD1a, GD1b, GD3, GT1b, and GQ1b each had no effect on IgG1 production at any concentration tested. GM2 and GM3 also inhibited production of IgG2 (Fig 1B), IgG3 (Fig 1C), IgG4 (Fig 1D), and IgM (Fig 1E) and thymidine uptake (Fig 1H). In contrast, GM2 and GM3 each had no effect on IgA1 or IgA2 production (Fig 1F and G). In 10 experiments attempted, the ranges of percent inhibition of IgG1 production by GM2 (1 \(\mu\)g/mL) and GM3 (1 \(\mu\)g/mL) were 52% to 83% and 55% to 88%, respectively, whereas those of percent inhibition of thymidine uptake by GM2 and GM3 were 60% to 82% and 56% to 81%, respectively. Similar degrees of inhibition were observed in the production of IgG2, IgG3, IgG4, and IgM by GM2 and GM3 (data not shown). In contrast, the ranges of percent inhibition of IgA1 production by GM2 and GM3 were 2% to 13% and 3% to 14%, respectively, whereas those of IgA2 production were, respectively, 1% to 11% and 2% to 9%.

---

**Fig 1.** Effects of gangliosides on B cells. Tonsillar B cells were cultured with SAC plus IL-2, and medium (○) or various concentrations of GM1 (□), GM2 (●), GM3 (△), GD1a (▲), GD1b (▼), GD3 (▼), GT1b (□), or GQ1b (■) were added. On day 7 of culture, production of IgG1 (A), IgG2 (B), IgG3 (C), IgG4 (D), IgM (E), IgA1 (F), and IgA2 (G) was determined. On day 5 of culture, thymidine uptake (H) was determined. B cells were also cultured with medium alone (☆) or SAC alone (⊙). Values are the means (n = 4). *\(p < .01\) compared with results for control cultures with medium.
GANGLIOSIDES INHIBIT HUMAN B-CELL RESPONSES

Fig 2. Kinetics of the effect of gangliosides. Tonsillar B cells were cultured with SAC plus IL-2 and medium (□), GM2 (1 μg/mL; □), or GM3 (1 μg/mL; □) was added. On days 3, 5, and 7 of culture, production of IgG1 (A), IgA1 (B), IgG2 (C), and IgA2 (D) and thymidine uptake (E) were determined. Values are the means ± SD (n = 4). *P < .01 compared with results for control cultures with medium.

Kinetics of the effects of gangliosides. As shown in Fig 2A, significant inhibition of IgG1 production by GM2 and GM3 was detected on day 3, with greatest inhibition noted on day 7. On the other hand, GM2 and GM3 each inhibited thymidine uptake maximally on day 5 (Fig 2B). In contrast, neither GM2 nor GM3 inhibited IgA1 or IgA2 production on any day tested (Fig 2C and D). Moreover, gangliosides must be added at the initiation of culture because they had no effect on IgG1 production and thymidine uptake if added 1 day after the culture (Table 1).

Specificity of GM2- and GM3-induced inhibition. As shown in Fig 3, the addition of neither anti-GM2 MoAb nor anti-GM3 MoAb had any effect on Ig production or thymidine uptake in the absence of GM2 or GM3. However, GM2- and GM3-induced inhibition of Ig production and thymidine uptake was blocked by anti-GM2 MoAb, but not by anti-GM3 MoAb. On the other hand, GM3-induced inhibition was blocked by anti-GM3 MoAb, but not by anti-GM2 MoAb. Similarly, GM2- and GM3-induced inhibitions were specifically blocked by rabbit anti-GM2 Ab and rabbit anti-GM3 Ab, respectively, but not by either control mouse IgM or control rabbit IgG (data not shown).

Effect of various factors on GM2- and GM3-induced inhibition. It is possible that GM2 and GM3 induce the production of known inhibitors for IL-2--stimulated B-cell responses, including TGF-β, IL-4, ECP, and PTH, as we and others have previously reported. Alternatively, GM2 and GM3 may inhibit endogenous production of B-cell stimulatory cytokines. As shown in Fig 4A and B, none of the antibodies to inhibitors blocked GM2- or GM3-induced inhibition. Addition of IL-1β, IL-3, IL-5, IL-6, and IFN-γ each enhanced control IgG1 production in the absence of gangliosides, whereas IL-1β, IL-3, and IFN-γ each enhanced control thymidine uptake. However, none of them affected GM2- or GM3-induced inhibition. IL-2 (100 U/mL) and IFN-α (1000 U/mL) each had no effect on inhibition (data not shown). In contrast, TNF-α not only enhanced control IgG1 production and thymidine uptake but also counteracted GM2- and GM3-induced inhibitions (Fig 4A and B). Similarly, TNF-α counteracted GM2- and GM3-induced inhibitions of production of IgG2, IgG3, IgG4, and IgM (data not shown). On the other hand, lymphotoxin and CD40L, related proteins to TNF-α, had no effect on inhibition. In three experiments, IgG1 production (in nanograms per milliliter) was 420 ± 56 by medium, 92 ± 13 by GM2, 94 ± 11 by GM2 plus lymphotoxin (100 ng/mL), 95 ± 10 by GM2 plus anti-CD40 MoAb (100 ng/mL), 115 ± 23 by GM3, 110 ± 16 by GM3 plus lymphotoxin, and 109 ± 12 by GM3 plus anti-CD40 MoAb. Similar results were observed for other Ig production and thymidine uptake by GM2/GM3 plus lymphotoxin or anti-CD40 MoAb (data not shown).

Specificity of the effect of TNF-α. As shown in Fig 5A and B, the addition of anti-TNF-α Ab inhibited control IgG1 production and thymidine uptake, indicating that endogenous TNF-α play a role in modulating B-cell responses. In contrast, anti-TNF-α Ab did not augment GM2- or GM3-induced inhibition. TNF-α counteracted the GM2- and GM3-induced inhibitions in a dose-dependent fashion, and this counteractive effect was blocked by anti-TNF-α Ab but not by control IgG. Identical counteractive effects of TNF-α were observed on GM2- and GM3-induced inhibition of production of IgG2, IgG3, IgG4, and IgM (data not shown).

Effects of GM2 and GM3 on purified slg+ B cells. It is

Table 1. Effect of Delayed Addition of Gangliosides

<table>
<thead>
<tr>
<th>Addition of the Gangliosides on</th>
<th>IgG1 (ng/mL)</th>
<th>CPM (×10^4)</th>
<th>IgG1 (ng/mL)</th>
<th>CPM (×10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition (control)</td>
<td>3.3</td>
<td>661</td>
<td>3.3</td>
<td>661</td>
</tr>
<tr>
<td>Day 0</td>
<td>141 (79)*</td>
<td>9.8 (73)*</td>
<td>123 (82)*</td>
<td>0.8 (76)*</td>
</tr>
<tr>
<td>Day 1</td>
<td>601 (9)</td>
<td>2.9 (12)</td>
<td>590 (11)</td>
<td>2.8 (15)</td>
</tr>
<tr>
<td>Day 2</td>
<td>658 (8)</td>
<td>3.0 (9)</td>
<td>636 (4)</td>
<td>2.9 (12)</td>
</tr>
<tr>
<td>Day 3</td>
<td>641 (9)</td>
<td>3.3 (0)</td>
<td>659 (0)</td>
<td>3.2 (3)</td>
</tr>
<tr>
<td>Day 4</td>
<td>669 (0)</td>
<td>3.1 (9)</td>
<td>664 (0)</td>
<td>3.4 (0)</td>
</tr>
</tbody>
</table>

In parentheses is the percent inhibition of control IgG1 production and thymidine uptake. Values are the means of triplicate cultures.

* P < .01 compared with control cultures with medium alone.
possible that GM2- and GM3-induced inhibitions were caused by decreased production of endogenous TNF-α and/or downregulation of IL-2 binding or TNF-α binding to B cells. We also attempted to determine the mechanisms responsible for the lack of inhibition of IgA1 or IgA2 production by gangliosides. For this purpose, B cells were separated into various Ig+ B-cell types, since Ig is produced by corresponding types of each of the Ig+ B cells under the conditions of culture used here. As shown in Fig 6, GM2 and GM3 each inhibited IgG1 production and thymidine uptake by IgG1+ B cells, but had no effect on IL-2 binding or TNF-α binding. However, GM2 and GM3 each markedly inhibited TNF-α production by IgG1+ B cells. GM2 and GM3 also had no effect on the expression of IL-2Rα5 or IL-2Rα7 on IgG1+ B cells (data not shown). Identical findings were obtained for IgG2+ and IgM+ B cells (Fig 6) and IgG3+ and IgG4+ B cells (data not shown). In contrast, neither GM2 nor GM3 inhibited IgA1 production or thymidine uptake or TNF-α production by IgA1+ B cells or IgA2+ B cells (Fig 6).

**Fig 3.** Specificity of the effect of gangliosides. Tonsillar B cells were cultured with SAC plus IL-2 in the presence of the indicated factors, and Ig production and thymidine uptake were determined. GM2 and GM3 were used at 1 μg/mL, and anti-GM2 MoAb and anti-GM3 MoAb at 10 μg/mL. Values are the means ± SD (n = 4) of results of four independent experiments, representative of 10. *P < .01 compared with results for control cultures with medium.

**Fig 4.** Effect of various factors on inhibition by gangliosides. Tonsillar B cells were cultured with SAC plus IL-2. They were incubated with medium, GM2, or GM3 in the presence or absence of the indicated factors, and IgG1 production (A) and thymidine uptake (B) were determined. GM2 and GM3 were used at 1 μg/mL, all the antibodies at 10 μg/mL, IL-1β at 100 U/mL, IL-3 at 100 U/mL, IL-5 at 100 ng/mL, IL-6 at 100 U/mL, IFN-γ at 1,000 U/mL, and TNF-α at 50 ng/mL. Values are the means ± SD (n = 4). *P < .01 compared with medium; **P < .01 compared with GM2 alone; ♠P < .01 compared with GM3 alone.
GANGLIOSIDES INHIBIT HUMAN B-CELL RESPONSES

Fig 5. Specificity of the effect of TNF-α on inhibition by gangliosides. Tonsillar B cells were cultured with SAC plus IL-2. They were incubated with medium, GM2, or GM3 in the presence or absence of the indicated factors, and IgG1 production (A) and thymidine uptake (B) were determined. GM2 and GM3 were used at 1 μg/mL, anti-TNF-α Ab at 10 μg/mL, and control IgG at 10 μg/mL. Values are the means ± SD (n = 4). * and tP < .01 compared with medium; **P < .01 compared with GM2 alone; t1P < .01 compared with GM3 alone.

Fig 6. Effects of gangliosides on purified slg+ B cells. Purified tonsillar slg+ B cells were cultured with SAC plus IL-2. They were incubated with medium, GM2 (1 μg/mL; □), or GM3 (1 μg/mL; ■), and Ig production, thymidine uptake, IL-2 binding, TNF-α binding, and TNF-α production were determined. Values are the means ± SD (n = 4). *P < .01 compared with results for control cultures with medium.
GM2 and GM3 inhibited TNF-α production by slgG1+, slgG2+, slgG3+, slgG4+, and slgM+ B cells in a dose-dependent fashion but not that by slgA1+ or slgA2+ B cells (Fig 7A through G). Moreover, GM2- and GM3-induced inhibitions of TNF-α production were blocked by anti-GM2 MoAb and anti-GM3 MoAb, respectively (Fig 7H); identical findings were obtained for slgG2+, slgG3+, slgG4+, and slgM+ B cells (data not shown). These findings indicate that GM2- and GM3-induced inhibition in slgG1+, slgG2+, slgG3+, slgG4+, and slgM+ B cells involved inhibition of TNF-α production but not downregulation of IL-2 binding or TNF-α binding. Because many of the IgA-producing cells in murine Peyer’s patches are CD5+, it is possible that GM2/GM3 resistance is related to the CD5 expression. However, this is unlikely because the percentage of CD5+ (Leu-1+) cells in slgA1+ and slgA2+ B cells is less than 1% (n = 3), in accordance with previous results of cord blood slgA+ B cells.29

The effects of GM2/GM3 were not confined to tonsillar B cells obtained by positive selection. Peripheral blood slgA−, slgA1+, and slgA2+ B cells were obtained by negative selection, and they were stimulated with high concentrations of IL-2 (300 U/mL) and IL-6 (100 U/mL). As shown in Table 2, they produced large amounts of Ig and proliferated vigorously. GM2/GM3 inhibited production of IgG (experiment no. 1) and IgM (experiment no. 2), thymidine uptake (experiments no. 1 and 2), and TNF-α production (experiment no. 1) by slgA− B cells. In contrast, GM2/GM3 had no effect on production of IgA1 and IgA2 by slgA1+ and slgA2+ B cells, respectively, and thymidine uptake and TNF-α production by them (Table 2).

**DISCUSSION**

We have shown that, of several gangliosides tested, only GM2 and GM3 inhibited production of IgG1, IgG2, IgG3, IgG4, and IgM, but not of IgA1 or IgA2, and thymidine uptake by human B cells. That gangliosides do differ in their effects depending on target cells is well known. For example, GM2 and GM3 have been found to inhibit Fcγ receptor expression but to have no effect on IL-1 production.18 In contrast, thymidine uptake by human peripheral blood lymphocytes is enhanced by GM2 but inhibited by GM3.30 The reasons for these differences in effects are not known in detail.

GM2- and GM3-induced inhibitions were specific, because they must be added at the initiation of the culture to achieve inhibition, which is in accordance with previous reports of MLC response.31 Moreover, their inhibitions were blocked by anti-GM2 and anti-GM3 antibodies, respectively. Furthermore, this inhibition was not mediated by known
inhibitors of IL-2–induced B-cell responses, including TGF-β, IL-4, ECP, and PTH, because antibodies to each of these failed to block inhibition. In addition, the known B-cell stimulatory cytokines IL-1β, IL-3, IL-5, IL-6, and IFN-γ each also had no effect on inhibition. In contrast, TNF-α did counteract GM2- and GM3-induced inhibition. It has been reported that gangliosides at high concentrations (>25 µg/mL) inhibited IL-2–induced T-cell proliferation by preventing binding of IL-2 to IL-2 receptors.12,30 However, GM2- and GM3-induced inhibitions of Ig production and thymidine uptake by B cells was not caused by inhibition of IL-2 binding, because neither GM2 nor GM3 at 1 µg/mL had any effect on IL-2 binding to B cells. Moreover, the addition of IL-2 also failed to counteract inhibition. GM2 and GM3 each also had no effect on TNF-α binding on B cells. In contrast, GM2 and GM3, but not other gangliosides tested, inhibited TNF-α production by slgG1–, slgG2–, slgG3–, slgG4–, and slgM– B cells; this inhibition was specifically blocked by anti-GM2 and anti-GM3 antibodies. However, GM2 and GM3 each failed to inhibit TNF-α production by slgA1– or slgA2– B cells; the reasons for the lack of effect of GM2 and GM3 on slgA1– and slgA2– B cells are currently under being studied in detail.

Our finding that gangliosides inhibit TNF-α production by B cells is not surprising. It has been reported that GM2 and GM3 inhibited TNF-α production by human monocytes,18,32 and tonsillar B cells produce TNF-α.33,34 Whether GM2/GM3 inhibit TNF-α mRNA, as they do in macrophages, and/or inhibit release of TNF-α from the cell membrane is currently under investigation. However, we measured cell-associated TNF-α by lysing B cells, and found that GM2/GM3 also decreased cell-associated TNF-α. In three experiments, cell-associated TNF-α (in nanograms per milliliter) in slgG1– B cells cultured with medium, GM2 (1 µg/mL), and GM3 (1 µg/mL) as 1.3 ± 0.3, 0.1 ± 0.1, and 0.2 ± 0.1, respectively. Similar findings were observed in slgG2–, slgG3–, slgG4–, and slgM– B cells (data not shown). These results indicate that GM2/GM3-induced inhibition of TNF-α production may not be simply caused by inhibition of release of TNF-α from the cell membrane. Taken together, these findings suggested that GM2- and GM3-induced inhibitions were caused by inhibition of endogenous TNF-α production. The effects of TNF-α on B-cell responses have been controversial. TNF-α enhanced B-cell responses in some studies, whereas it was either without effect on or inhibited B-cell responses in others. This discrepancy in findings may be caused by differences in stimulation or cell sources, eg, tonsil versus blood, as previously reported.23,67 Our results are in accord with those of Rodriguez et al.,9 who found that TNF-α was essential for B-cell responses by tonsillar B cells. The in vivo role of GM2 and GM3 in modulating B-cell responses remains to be elucidated. However, Ig production and thymidine uptake in vitro by B cells from patients with GM2 gangliosidosis were less than those by normal donors’ B cells. The addition of TNF-α restored Ig production and thymidine uptake by patients’ B cells to normal levels (our unpublished observation). Taken together, these findings suggest that gangliosides are useful for the study of B-cell responses.

ACKNOWLEDGMENT

We thank Takeda Chemical Industries (Osaka, Japan) for kindly providing the human recombinant IL-2, IFN-α, and IFN-γ used in this study, and for their continuing encouragement of our research.

REFERENCES


Table 2. Effects of Gangliosides on Peripheral Blood slgA– and slgA+ B Cells

<table>
<thead>
<tr>
<th>B Cells</th>
<th>Factors</th>
<th>Experiment No. 1</th>
<th></th>
<th>Experiment No. 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ig (µg/mL)</td>
<td>CPM (×10⁴)</td>
<td>TNF-α (ng/mL)</td>
<td>Ig (µg/mL)</td>
</tr>
<tr>
<td>slgA–</td>
<td>Medium</td>
<td>23 ± 3</td>
<td>36 ± 6</td>
<td>14 ± 2</td>
<td>32 ± 4</td>
</tr>
<tr>
<td></td>
<td>GM2</td>
<td>4 ± 1*</td>
<td>6 ± 1*</td>
<td>1 ± 1*</td>
<td>7 ± 2*</td>
</tr>
<tr>
<td></td>
<td>GM3</td>
<td>5 ± 1*</td>
<td>8 ± 2*</td>
<td>1 ± 1*</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>slgA1+</td>
<td>Medium</td>
<td>21 ± 4</td>
<td>33 ± 6</td>
<td>12 ± 2</td>
<td>29 ± 6</td>
</tr>
<tr>
<td></td>
<td>GM2</td>
<td>19 ± 5</td>
<td>31 ± 6</td>
<td>11 ± 2</td>
<td>31 ± 4</td>
</tr>
<tr>
<td></td>
<td>GM3</td>
<td>22 ± 4</td>
<td>32 ± 4</td>
<td>13 ± 3</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>slgA2–</td>
<td>Medium</td>
<td>19 ± 4</td>
<td>24 ± 5</td>
<td>14 ± 3</td>
<td>25 ± 6</td>
</tr>
<tr>
<td></td>
<td>GM2</td>
<td>21 ± 5</td>
<td>22 ± 4</td>
<td>12 ± 3</td>
<td>23 ± 4</td>
</tr>
<tr>
<td></td>
<td>GM3</td>
<td>19 ± 3</td>
<td>25 ± 7</td>
<td>15 ± 4</td>
<td>26 ± 4</td>
</tr>
</tbody>
</table>

Peripheral blood slgA–, slgA1+, and slgA2+ B cells were cultured with SAC (0.01%), IL-2 (300 U/mL), and IL-6 (100 U/mL). They were incubated with medium, GM2 (1 µg/mL), or GM3 (1 µg/mL), and Ig production, thymidine uptake, and TNF-α production were determined.

* P < .01 compared with results for control cultures with medium.
and proliferation in serum-free medium. Acta Endocrinol 126:524, 1992
Differential effects of gangliosides on Ig production and proliferation by human B cells

H Kimata and A Yoshida