B-cell-stimulatory factor 1 reverses Fc receptor-mediated inhibition of B-lymphocyte activation
(interleukin 4/antibody feedback/anti-Fab fragment antibodies)

Anne O’Garra*, Kevin P. Rigley*, Mary Holman*, John B. McLaughlin†, and Gerry G. B. Klaus*

*Division of Immunology, National Institute for Medical Research, London NW7 1AA; and †Glaxo Group Research, Greenford, Middlesex, United Kingdom

Communicated by J. H. Humphrey, May 21, 1987 (received for review March 26, 1987)

ABSTRACT Intact (IgG class) rabbit anti-immunoglobulin antibodies are not mitogenic for mouse B cells but inhibit proliferation induced by F(ab′)2 anti-Fab fragment antibodies (anti-Ig). In addition, cross-linkage of Fc and surface immunoglobulin receptors on B cells by intact anti-Ig inhibits inositol phospholipid breakdown (but not Ca2+ flux) resulting from ligation of sIg receptors. This system, therefore, provides a polyclonal model for B-cell inactivation by antigen–antibody complexes. T-cell-derived B-cell-stimulatory factor 1 acts synergistically with submitogenic concentrations of F(ab′)2 anti-Ig to induce B-cell proliferation. We show here that B-cell-stimulatory factor 1 and intact anti-Ig also induce B cells to synthesize DNA. However, B-cell-stimulatory factor 1 does not induce inositol phospholipid breakdown, does not mobilize Ca2+ in B cells, nor does it influence the magnitude of these responses provoked by intact anti-Ig.

MATERIALS AND METHODS

Mice. Three- to six-month-old male (CBA × C57BL/6)F1 mice, bred under specific-pathogen-free conditions at the National Institute for Medical Research (London) were used.

Reagents. The preparation of affinity-purified intact IgG, F(ab′)2, and Fab fragments of rabbit anti-mouse Fab antibodies (henceforth called anti-Ig) has been described (9). 2-[3H]Inositol (15 Ci/mmol; 1 Ci = 37 GBq), 5-[3H]Uridine (2–5 Ci/mmol), and 3H[thymidine (5 Ci/mmol) were from Amersham. Indo-1 acetoxyethyl ester was from Molecular Probes (Piano, TX).

Preparation of B Cells. These were prepared from spleens as described elsewhere (9, 19). Small, dense B cells were taken from the 85%/75% interface of two consecutive discontinuous Percoll (Pharmacia) density gradients (20). In some experiments, they were additionally filtered through Sephadex G-10 to remove adherent cells (9). For biochemical analyses, B cells were taken from the 85%/65% interface of Percoll density gradients and routinely filtered through Sephadex G-10 (9). The latter preparations contained >90% slg-positive cells.

Lymphokine Preparations. Conditioned medium (CM-T2), from an allosreactive T-cell clone NIMP-T2 (21), was used in most experiments. This contains high titers of BSF-1 and BCGF-II and low levels of interleukins 2 and 3. Additional experiments were performed using highly purified (22) preparations of BSF-1, kindly provided by J. Ohara and W. E. Paul (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD), or recombinant BSF-1 (23) donated by M. J. Howard (DNAX Research Institute, Palo Alto, CA). Partially purified BCGF-II was from the conditioned medium of a T-cell hybrid NIMP-TH1, which produces no other detectable lymphokines (21, 24). A unit of BSF-1 is defined as the dilution of factor required to give half-maximal DNA synthesis with submitogenic doses of the F(ab′)2 fragment of anti-Ig (22). Similarly, a unit of BCGF-II is the dilution of factor required to give half-maximal proliferation of the BCL1 lymphoma (25).

B-Cell Culture Systems. Aliquots of 5 × 10^5 cells were cultured in flat-bottomed microtiter wells in 200 μl of supplemented RPMI 1640 medium (9) containing 5% (vol/vol) of FCS. The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
fetal calf serum. DNA synthesis was determined by adding \[^{3}H\]thymidine (0.5 \(\mu\)Ci per well) to cultures after 68 hr and harvesting after a 4-hr labeling period. Two-stage priming cultures to measure entry of resting B cells into the cell cycle were done as described (9).

**Assay of Inositol Phospholipid Breakdown.** B cells were labeled with \[^{3}H\]inositol (0.5 \(\mu\)Ci per 10^6 cells) (26). In some experiments aliquots were preincubated at 37°C for 30 min with or without CM-T2 in complete medium containing 5 mM LiCl. The cells were then incubated with anti-Ig for 60 min, and total amounts of inositol phosphates were determined (26). In other experiments B cells were preincubated with CM-T2 for 24 hr at 37°C. They were then resuspended in inositol-free medium in the presence or absence of CM-T2, labeled with \[^{3}H\]inositol (1 \(\mu\)Ci per 10^6 cells), and stimulated with anti-Ig as above.

**Determination of Free Intracellular Ca^{2+} Levels ([Ca^{2+}]_i).** Cells were incubated with 2 \(\mu\)M indo-1 acetoxyethyl ester (26, 27). In some experiments, indo-1-loaded cells were washed and resuspended to 5 \times 10^6 cells per ml in Heps-buffered Hanks’ solution, pH 7.3, either with or without CM-T2 [dialyzed against isotonic phosphate-buffered saline (PBS)]. After 30 min at 37°C, the cells were washed and [Ca^{2+}]_i increases in response to anti-Ig were measured by spectrofluorimetry (27). In other experiments [Ca^{2+}]_i levels of indo-1-loaded B cells, stimulated with the F(ab')2 fragment of anti-Ig or intact anti-Ig and with CM-T2, were determined. Alternatively, B cells were preincubated with CM-T2 or medium for 24 hr, loaded with indo-1, and then restimulated with the F(ab')2 fragment of anti-Ig or PBS.

**Cell Cycle Analyses.** Cells for flow cytometric analysis were kept in Hanks’ balanced salts solution containing 0.5% bovine serum albumin at 4°C. They were stained with acridine orange and ethidium bromide to final concentrations of 15 \(\mu\)M and 3 \(\mu\)M, respectively (28, 29), and analyzed on a Cytofluorograf system 50H (Ortho Diagnostic Systems, Westwood, MA). The excitation wavelength has 488 nm with collection of green fluorescence at 530–565 nm and red fluorescence at >640 nm (29, 30).

**RESULTS**

**T-Cell Factor(s) Act Synergistically with the F(ab')2 Fragment of Anti-Ig and Intact Anti-Ig.** High concentrations of the bivalent Fab fragment or the intact IgG of anti-Ig, but not the monomeric Fab fragment or the intact IgG of anti-Ig, induce significant levels of DNA synthesis in mouse B cells (Fig. 1). Furthermore, CM-T2, which contains BSF-1, markedly potentiated the response to submitogenic concentrations of F(ab')2 anti-Ig (Fig. 1A). More importantly, B cells cultured with intact anti-Ig plus CM-T2 also showed substantial levels of DNA synthesis (Fig. 1B).

These results, therefore, indicate that one or more T-cell-derived lymphokines enable the nonmitogenic intact form of anti-Ig to induce B-cell proliferation. However, these factors do not overcome the requirement for slg cross-linking, since CM-T2 did not act synergistically with Fab fragments of anti-Ig (Fig. 1C). The results in Fig. 1 also suggest that fewer B cells synthesize DNA in response to intact anti-Ig plus BSF-1 than to the F(ab')2 antibodies plus the factor. To study this further we monitored RNA and DNA levels in B cells by flow cytometric analyses of acridine orange-stained cells. After 70 hr in culture, some 30% of cells stimulated with CM-T2 plus intact anti-Ig were in S phase compared with 70% of those given the factor(s) plus F(ab')2 anti-Ig.

It is, therefore, clear that T-cell factor(s) cannot overcome FcR-mediated growth inhibition in all B cells, so that a fraction of those cultured with intact anti-Ig remain blocked in the Go/G1 phase of the cycle.

**Purified BSF-1 Acts Synergistically with Both F(ab')2 and Intact Anti-Ig.** Since CM-T2 contains both BSF-1 and BCGF-II, we next tested the capacity of the purified factors to induce proliferation with anti-Ig. Table 1 confirms that highly purified BSF-1, derived from the EL4 cell line (22), and recombinant BSF-1 (23) costimulate DNA synthesis with F(ab')2 anti-Ig. Significantly, both preparations of BSF-1 evoked the same level of DNA synthesis with intact anti-Ig as CM-T2. This strongly suggests that BSF-1 is the only factor in the conditioned medium responsible for this effect. In marked contrast, partially purified BCGF-II did not affect stimulation of small, dense B cells by F(ab')2 (21) or intact anti-Ig (Table 1).

**BSF-1 Is Required as an Early Costimulus with Anti-Ig to Cause B-Cell Proliferation.** To investigate at what stage in the costimulator system BSF-1 is required, B cells were incubated with CM-T2 and with equimolar amounts of IgG or F(ab')2 anti-Ig. In Fig. 2A, the CM-T2 was added at different
Table 1. Costimulation of B cells with purified or recombinant BSF-1 and F(ab')2 or intact anti-Ig

<table>
<thead>
<tr>
<th>Addition to culture</th>
<th>[3H]Thymidine uptake, cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG anti-Ig</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>CM-T2</td>
<td>16,800 ± 1200</td>
</tr>
<tr>
<td>BSF-1 (2 units/ml)</td>
<td>16,000 ± 1700</td>
</tr>
<tr>
<td>BSF-1 (10 units/ml)</td>
<td>12,700 ± 680</td>
</tr>
<tr>
<td>BSF-1 (5 units/ml)</td>
<td>10,100 ± 1500</td>
</tr>
<tr>
<td>Medium alone</td>
<td>530 ± 40</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>CM-T2</td>
<td>6,700 ± 240</td>
</tr>
<tr>
<td>BSF-1 (26 units/ml)</td>
<td>6,200 ± 680</td>
</tr>
<tr>
<td>BSF-1 (5 units/ml)</td>
<td>5,600 ± 160</td>
</tr>
<tr>
<td>BSF-1 (2.5 units/ml)</td>
<td>4,500 ± 100</td>
</tr>
<tr>
<td>BSF-1 (0.25 units/ml)</td>
<td>3,200 ± 300</td>
</tr>
<tr>
<td>BCGF-II (2 units/ml)</td>
<td>84 ± 20</td>
</tr>
<tr>
<td>BCGF-II (1 unit/ml)</td>
<td>160 ± 20</td>
</tr>
<tr>
<td>Medium alone</td>
<td>380 ± 60</td>
</tr>
</tbody>
</table>

In experiment 1, small, dense B cells were cultured with various combinations of CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml] or highly purified EL4-derived BSF-1 and intact IgG anti-Ig (10 μg/ml) or the F(ab')2 fragment of anti-Ig (6.6 μg/ml). In experiment 2, B cells were cultured with CM-T2 (same concentration), with recombinant BSF-1, or with semipurified BCGF-II and with intact IgG anti-Ig (10 μg/ml) or the F(ab')2 fragment of anti-Ig (6.6 μg/ml). [3H]Thymidine uptakes were measured after 72 hr in culture and are means ± SEM of triplicate cultures.

Times after initiation of the incubation with antibody, while in Fig. 2B, the time of addition of anti-Ig was varied. The results indicate that delaying the addition of BSF-1 to F(ab')2 anti-Ig or to intact anti-Ig-stimulated cultures by >10 hr, substantially reduced levels of DNA synthesis. In contrast, delaying the addition of anti-Ig to cells stimulated with BSF-1 had little effect until >20 hr. Therefore, an early and prolonged stimulation of B cells with BSF-1 is necessary to obtain a costimulator effect with both forms of anti-Ig.

**BSF-1-Activated B Cells Do Not Synthesize DNA When Restimulated by Intact Anti-Ig Alone.** It has been shown (21, 31, 32) that BSF-1 primes resting B cells to synthesize DNA more rapidly when they are recultured with a mitogenic preparation of anti-Ig. We, therefore, tested whether BSF-1-activated B cells would also respond to intact anti-Ig. B cells were cultured with CM-T2 or F(ab')2 anti-Ig for 24 hr, washed, and restimulated with F(ab')2 anti-Ig or with intact anti-Ig (Fig. 3). As expected, cells primed with F(ab')2 anti-Ig or with CM-T2 responded well to F(ab')2 anti-Ig. However, they did not respond to restimulation by the intact antibodies. These results indicate that BSF-1 can only reverse the inhibitory effects of intact anti-Ig when present at the same time as the antibody.

**BSF-1 Does Not Affect Anti-Ig-Induced Inositol Phospholipid Breakdown.** F(ab')2 anti-Ig stimulates rapid breakdown of PtdInsP2 in B cells, resulting in prolonged release of inositol phosphates and diacylglycerol (26). In marked contrast, intact anti-Ig induces an abortive response, which is due to binding of this ligand to FcR (10). Since it is likely that anti-Ig-stimulated PtdInsP2 breakdown is involved in induction of B-cell activation, we studied the effects of BSF-1 on inositol phosphate release provoked by the two forms of anti-Ig.

In initial experiments aliquots of [3H]inositol-labeled B cells in medium with 5 mM Li⁺ were preincubated for 30 min with or without CM-T2. The cells then received either F(ab')2 or IgG anti-Ig, with or without CM-T2, and total inositol phosphate levels were determined 1 hr later. As shown in Fig. 4A, preincubation of B cells with BSF-1 did not affect the barely detectable release of [3H]inositol phosphates induced by intact anti-Ig.

Since an early but prolonged stimulation of B cells with BSF-1 appears necessary to obtain a costimulatory effect with anti-Ig, we next studied the effects of preincubating B cells with CM-T2 for 24 hr. Again, this did not influence the response to IgG anti-Ig (data not shown).

**BSF-1 Does Not Affect the Increase in [Ca²⁺], Induced by F(ab')2 or Intact Anti-Ig.** Both F(ab')2 and IgG anti-Ig elicit comparable increases in [Ca²⁺], in B cells (10), which are initially due to release from intracellular stores and then due
The cells then received CM-T2, and total IgG antibodies, because they cocross-link slg and FcR on these cells (4). This generates a powerful inhibitory signal, the nature of which is unknown, but is presumably delivered via the FcR. The present results show that T-cell-conditioned medium acts synergistically with intact anti-Ig to induce DNA synthesis in a substantial fraction of B cells and that the lymphokine responsible is BSF-1 (Fig. 1 and Table 1). Furthermore, this effect requires the continuous presence of the factor. Thus, cells preactivated by BSF-1 or F(ab')2 anti-Ig do not synthesize DNA when recultured with intact anti-Ig alone (Fig. 3). However, it is also evident that BSF-1 does not overcome the inhibitory effects of intact anti-Ig in all B cells, since substantially more cells enter S phase in cultures stimulated with F(ab')2 anti-Ig plus BSF-1.

The effects of intact anti-Ig on B cells appear to mimic those of antigen-antibody complexes (4). There is also a large body of evidence supporting the concept of Fc-dependent inactivation of B cells in a wide variety of experimental systems, including IgG antibody-mediated feedback (35), the inhibitory effects of anti-idiotypic antibodies (3), and tolerance induction by immunoglobulin antigens (36). These phenomena have been explained by the tripartite inactivation model (37), which proposes that cocross-linking of antigen receptors and FcR by appropriate ligands generates inhibitory signals through the latter.

**DISCUSSION**

It is well established that intact rabbit anti-mouse immunoglobulin antibodies are not mitogenic for B cells but instead inhibit DNA synthesis induced by F(ab')2 antibodies, because they cocross-link slg and FcR on these cells (4). This generates a powerful inhibitory signal, the nature of which is unknown, but is presumably delivered via the FcR. The present results show that T-cell-conditioned medium acts synergistically with intact anti-Ig to induce DNA synthesis in a substantial fraction of B cells and that the lymphokine responsible is BSF-1 (Fig. 1 and Table 1). Furthermore, this effect requires the continuous presence of the factor. Thus, cells preactivated by BSF-1 or F(ab')2 anti-Ig do not synthesize DNA when recultured with intact anti-Ig alone (Fig. 3). However, it is also evident that BSF-1 does not overcome the inhibitory effects of intact anti-Ig in all B cells, since substantially more cells enter S phase in cultures stimulated with F(ab')2 anti-Ig plus BSF-1.

The effects of intact anti-Ig on B cells appear to mimic those of antigen-antibody complexes (4). There is also a large body of evidence supporting the concept of Fc-dependent inactivation of B cells in a wide variety of experimental systems, including IgG antibody-mediated feedback (35), the inhibitory effects of anti-idiotypic antibodies (3), and tolerance induction by immunoglobulin antigens (36). These phenomena have been explained by the tripartite inactivation model (37), which proposes that cocross-linking of antigen receptors and FcR by appropriate ligands generates inhibitory signals through the latter.

**FIG. 3.** BSF-1-activated B cells do not synthesize DNA in response to intact anti-Ig. Small, dense B cells were cultured at 10⁶ cells per ml with CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml], F(ab')2 anti-Ig (10 μg/ml), or medium alone. After 24 hr the cells were washed and cultured at 5 × 10⁶ viable cells per 200 μl with F(ab')2 or intact anti-Ig at the indicated concentrations (in μg/ml). [³H]Thymidine uptake was determined after a further 24 hr. Results are means ± SEM of triplicate cultures.

The effects of intact anti-Ig on B cells appear to mimic those of antigen-antibody complexes (4). There is also a large body of evidence supporting the concept of Fc-dependent inactivation of B cells in a wide variety of experimental systems, including IgG antibody-mediated feedback (35), the inhibitory effects of anti-idiotypic antibodies (3), and tolerance induction by immunoglobulin antigens (36). These phenomena have been explained by the tripartite inactivation model (37), which proposes that cocross-linking of antigen receptors and FcR by appropriate ligands generates inhibitory signals through the latter.

**FIG. 4.** BSF-1 does not affect inositol phospholipid breakdown or the [Ca²⁺]i increase induced by anti-Ig. (A) Aliquots of [³H]inositol-labeled B cells, in medium with 5 mM Li⁺, were preincubated with or without CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml] for 30 min. The cells then received either F(ab')2 or intact anti-Ig at the concentrations indicated (in μg/ml), with or without the same concentration of CM-T2, and total [³H]inositol phosphate levels were determined 1 hr later. The results are given as percentages of the total cellular radioactivity (23,260 dpm on average). (B) Increases in [Ca²⁺]i in indo-1-loaded B cells were measured after the following additions. n, F(ab')2 anti-Ig at 50 μg/ml. o, F(ab')2 anti-Ig at 6.6 μg/ml. 0, F(ab')2 anti-Ig at 6.6 μg/ml with CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml]. ©, IgG anti-Ig at 10 μg/ml. •, IgG anti-Ig at 10 μg/ml with CM-T2. ◇, CM-T2 alone. ▲, Medium alone. Stimuli were added at the time indicated by the arrow.

**FIG. 3.** BSF-1-activated B cells do not synthesize DNA in response to intact anti-Ig. Small, dense B cells were cultured at 10⁶ cells per ml with CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml], F(ab')2 anti-Ig (10 μg/ml), or medium alone. After 24 hr the cells were washed and cultured at 5 × 10⁶ viable cells per 200 μl with F(ab')2 or intact anti-Ig at the indicated concentrations (in μg/ml). [³H]Thymidine uptake was determined after a further 24 hr. Results are means ± SEM of triplicate cultures.

The effects of intact anti-Ig on B cells appear to mimic those of antigen-antibody complexes (4). There is also a large body of evidence supporting the concept of Fc-dependent inactivation of B cells in a wide variety of experimental systems, including IgG antibody-mediated feedback (35), the inhibitory effects of anti-idiotypic antibodies (3), and tolerance induction by immunoglobulin antigens (36). These phenomena have been explained by the tripartite inactivation model (37), which proposes that cocross-linking of antigen receptors and FcR by appropriate ligands generates inhibitory signals through the latter.

**DISCUSSION**

It is well established that intact rabbit anti-mouse immunoglobulin antibodies are not mitogenic for B cells but instead inhibit DNA synthesis induced by F(ab')2 antibodies, because they cocross-link slg and FcR on these cells (4). This generates a powerful inhibitory signal, the nature of which is unknown, but is presumably delivered via the FcR. The present results show that T-cell-conditioned medium acts synergistically with intact anti-Ig to induce DNA synthesis in a substantial fraction of B cells and that the lymphokine responsible is BSF-1 (Fig. 1 and Table 1). Furthermore, this effect requires the continuous presence of the factor. Thus, cells preactivated by BSF-1 or F(ab')2 anti-Ig do not synthesize DNA when recultured with intact anti-Ig alone (Fig. 3). However, it is also evident that BSF-1 does not overcome the inhibitory effects of intact anti-Ig in all B cells, since substantially more cells enter S phase in cultures stimulated with F(ab')2 anti-Ig plus BSF-1.

The effects of intact anti-Ig on B cells appear to mimic those of antigen-antibody complexes (4). There is also a large body of evidence supporting the concept of Fc-dependent inactivation of B cells in a wide variety of experimental systems, including IgG antibody-mediated feedback (35), the inhibitory effects of anti-idiotypic antibodies (3), and tolerance induction by immunoglobulin antigens (36). These phenomena have been explained by the tripartite inactivation model (37), which proposes that cocross-linking of antigen receptors and FcR by appropriate ligands generates inhibitory signals through the latter.

**FIG. 4.** BSF-1 does not affect inositol phospholipid breakdown or the [Ca²⁺]i increase induced by anti-Ig. (A) Aliquots of [³H]inositol-labeled B cells, in medium with 5 mM Li⁺, were preincubated with or without CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml] for 30 min. The cells then received either F(ab')2 or intact anti-Ig at the concentrations indicated (in μg/ml), with or without the same concentration of CM-T2, and total [³H]inositol phosphate levels were determined 1 hr later. The results are given as percentages of the total cellular radioactivity (23,260 dpm on average). (B) Increases in [Ca²⁺]i in indo-1-loaded B cells were measured after the following additions. n, F(ab')2 anti-Ig at 50 μg/ml. o, F(ab')2 anti-Ig at 6.6 μg/ml. 0, F(ab')2 anti-Ig at 6.6 μg/ml with CM-T2 [5% (vol/vol) containing 5 units of BSF-1 per ml]. ©, IgG anti-Ig at 10 μg/ml. •, IgG anti-Ig at 10 μg/ml with CM-T2. ◇, CM-T2 alone. ▲, Medium alone. Stimuli were added at the time indicated by the arrow.
Earlier work had shown that T cells or T-cell replacing factor(s) can reverse Fc-dependent IgG antibody feedback (11–13). Since BSF-1 can substantially reverse the Fc-dependent inhibitory effects of anti-Ig, it now remains to be established if this factor can similarly influence Fc-dependent feedback of specific antibody responses. The fact that BGCF-II (which does have T-cell-replacing factor activity; ref. 17 and 18) does not act synergistically with intact anti-Ig (Table 1) was predictable, since this lymphokine acts on precipitated B cells (21). Indeed, preliminary experiments have shown that intact anti-Ig inhibits BGCF-II-induced DNA synthesis in large, naturally occurring B cells.

We have shown that intact anti-Ig induces abortive activation of resting B cells driving the cells into a transitional state (G1), characterized by the expression of high levels of Ia antigens (9). Unlike the F(ab')2 antibodies, the intact form provokes a short-lived breakdown of PtdInsP2 in B cells (10) that, however, appears to be sufficient to generate a normal increase in [Ca2+]i. Since it is likely that PtdInsP2 breakdown and the resulting increase in [Ca2+]i, provoked by F(ab')2-anti-Ig are involved in B-cell activation, we postulated that the [Ca2+]i increase provoked by intact anti-Ig is sufficient to explain the abortive activation induced by this ligand.

Our present results show that, although BSF-1 costimulates DNA synthesis with intact anti-Ig, the factor does not alter the release of inositol phosphates or the magnitude of the [Ca2+]i increase stimulated by these antibodies under a wide variety of experimental conditions (Fig. 4). This suggests that BSF-1 does not decrease the numbers of FcR on B cells significantly, although this has not been studied directly. In addition, CM-T2 alone neither provoked detectable PtdInsP2 breakdown nor caused an increase in [Ca2+]i, and that, at least in the short term, the factor does not detectably affect signaling by way of Ig receptors (see also refs. 33 and 34). Although the mechanisms whereby FcR inhibit B-cell activation remain to be elucidated, the simplest explanation of our present findings is that the low levels of PtdInsP2 breakdown provoked by intact anti-Ig are sufficient to provide the necessary costimulus for BSF-1-induced DNA synthesis. This is supported by studies on nonmitogenic anti-Ig monoclonal antibodies, some of which stimulate minimal PtdInsP2 hydrolysis and yet act synergistically and effectively with BSF-1 (K.P.R., unpublished data).

We thank Drs. J. Ohara, W. E. Paul, M. J. Howard, and K. Ari for their generous gifts of BSF-1; and Drs. C. J. Sanderson and D. J. Warren for providing purified BGCF-II. In addition, we thank Dr. David Scott for his positive intellectual input to the writing of the paper. A.O.G. was supported by a fellowship from Glaxo Group Research, and K.P.R. was supported by a studentship from the Wellcome Trust.