Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8766–8770, November 1990
Immunology

Ligation of membrane immunoglobulin leads to inactivation of the signal-transducing ability of membrane immunoglobulin, CD19, CD21, and B-cell gp95

(IgM/IgD/CD19/CD21/transmembrane signaling)

GER T. RIJKERS*†, ARJAN W. GRIFFIOEN*, BEN J. M. ZEGERS*, AND JOHN C. CAMBIER†‡

*Department of Immunology, University Hospital for Children and Youth, "Het Wilhelmina Kinderziekenhuis," Utrecht, The Netherlands; and †Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

Communicated by John W. Kappler, July 13, 1990

ABSTRACT **We have examined the ability of membrane immunoglobulin-binding ligands to desensitize several human B-cell surface molecules that normally transduce signals leading to Ca^{2+} mobilization. Ligation of membrane IgM or IgD leads to heterologous desensitization of the reciprocal receptor in Epstein–Barr virus-transformed B-cell lines and peripheral blood B cells, as evidenced by a failure of cells to mobilize in response to receptor ligation. Under these conditions CD19, CD21, and B-cell gp95 ligation also did not lead to normal Ca^{2+} mobilization, indicating that these transducers are also desensitized. The desensitization does not reflect receptor modulation from the cell surface or reduced accessibility to ligand and is long lived, lasting >16 hr. Finally, data that indicate that desensitized cells remain responsive to the G protein activating agent AlF_4^-, as measured by Ca^{2+} mobilization, suggest that desensitization reflects uncoupling of these receptors from G proteins that are intermediaries in their transduction of signals. We hypothesize that the molecular target of desensitization may be a recently described membrane immunoglobulin-associated and inducibly tyrosine-phosphorylated protein complex that may function as a master transducer in B cells, analogous to CD3 in T cells.**

Ligation of the antigen receptors [membrane immunoglobulin (mIg)] on B cells leads, via GTP-binding protein activation (1–3) to hydrolysis of phosphatidylinositol 4,5-bisphosphate by a phospholipase C (PLC), generating diacylglycerol and inositol 1,4,5-trisphosphate (for review, see ref. 4). Inositol 1,4,5-trisphosphate mediates the release of intracellular stored Ca^{2+} into the cytoplasm: the influx from extracellular Ca^{2+} is probably mediated by inositol 1,3,4,5-tetrakisphosphate and inositol 1,4,5-trisphosphate (5). Diacylglycerol stimulates the translocation and activation of protein kinase C (6). This cascade is causally linked to increased expression of genes encoding c-fos, c-myc, and Ia and to B-lymphocyte proliferation (4, 7).

Under certain circumstances, stimulation of murine B cells with antigen or anti-μ (membrane IgM) or anti-δ (membrane IgD) antibodies induces a state in which the remaining unligated antigen receptors of the cells are unable to transduce signals leading to increased intracellular Ca^{2+} $[Ca^{2+}]_i$ or protein kinase C translocation (8, 9). This desensitization may be the basis of antigen-induced clonal anergy (10, 11). This ligand-induced unresponsive state is not mediated by protein kinase C and appears to reflect uncoupling of mIg from its G protein. It is unclear whether ligand-induced desensitization affects only antigen receptor function or whether other cell-surface transducers are also desensitized. This is an important question because it has significant implications for the responsiveness of the desensitized cells to other ligands.

In human B cells a number of nonimmunoglobulin cell-surface molecules have been described that transduce signals leading to Ca^{2+} mobilization. These molecules include CD19, a 90-kDa pan-B cell determinant with unknown function (12), CD21, the 145-kDa complement receptor type 2 (ref. 13 and A.W.G., G.T.R., and B.J.M.Z., manuscript in preparation), and B-cell gp95 (Bgp95), a recently identified determinant present on quiescent B cells (14). The ability to activate these tranducers provides the opportunity to determine the specificity of anti-immunoglobulin-induced desensitization.

Here we describe a series of experiments conducted using human B cells that show that anti-μ not only induces desensitization of mIg but also renders CD19, CD21, and Bgp95 incapable of transducing Ca^{2+} mobilizing signals.

MATERIALS AND METHODS

Cells and Reagents. Heparinized venous blood was obtained from healthy adult volunteers. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation over Ficoll-Isopaque (Pharmacia). T cells were removed from peripheral blood mononuclear cells by rosetting with 2-aminoethylisothouronium bromide (AET)-treated sheep erythrocytes, followed by density-gradient centrifugation. Human Burkitt lymphoma (BL) cell lines were grown in RPMI 1640 medium/10% fetal calf serum.

$F(ab')_2$ fragments of a goat anti-human IgM antiserum were obtained from Organon Teknika–Cappel; murine monoclonal anti-human IgM (2C3) and anti-Bgp95 (G28-8) were donated by E. Clark (Seattle). Ionomycin was from Calbiochem.

Fluorescence Measurement of Intracellular Calcium and Marker Expression. Procedures used for loading cells with indo-1/AM (Molecular Probes) and flow cytometric analysis of changes in $[Ca^{2+}]_i$ have been described (15, 16). Briefly, cells were loaded with 4 μM indo-1/AM and analyzed using a fluorescein-activated cell sorter analyzer equipped with a mercury arc lamp as a UV light source.

For immunofluorescence, cells were washed and resuspended in modified Eagle's medium (MEM)/1% bovine serum albumin (BSA)/0.05% sodium azide (MEM/BSA/azide). Cells ($0.5–1 \times 10^6$) were incubated with gentle shaking for various periods at 4°C in 10 μl of appropriately diluted goat anti-human IgD (Tago), CD19 (Becton Dickinson), CD20 (B1; Coulter), CD21 (BD), or anti-Bgp95 and washed three times with ice-cold MEM/BSA/azide. Cells were then

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.

Abbreviations: mIg, membrane immunoglobulin; PLC, phospholipase C; Bgp95, B-cell gp95; $[Ca^{2+}]_i$, intracellular Ca^{2+}.

‡To whom reprint requests should be addressed at: Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

incubated with fluoresceinated goat anti-mouse immunoglobulin (BD) 30 min at 4°C and analyzed on a fluorescein-activated cell sorter analyzer (Becton Dickinson). Ten thousand events were stored per sample in list mode and analyzed by using Consort 30 (Becton Dickinson) software.

RESULTS

Stimulation of Human B Cells with Anti-μ Antibodies Induces Unresponsiveness to Subsequent Stimulation with Anti-δ Antibodies. BLgpt1 is a human Epstein–Barr virus-transformed B-cell line that expresses both surface μ and surface δ at high density (17). Incubation of BL cells with polyclonal or monoclonal antibody reagents leads to a rapid, but transient, increase in $[Ca^{2+}]_i$. This increase occurs within 15 sec after adding anti-μ. At maximal $[Ca^{2+}]_i$, which is 45–60 sec after stimulation, all cells display increased $[Ca^{2+}]_i$ (Fig. 1). Stimulation of BL cells with anti-δ reagents can also increase $[Ca^{2+}]_i$, but in this case only ≈40% of the cells respond. Magnitude of the anti-δ-induced calcium response is lower than the anti-μ response (Fig. 1). Consistent with the findings of Cambier *et al.* (8) and Harnett *et al.* (9) in murine B cells, the anti-δ response was completely abolished when BL cells were previously treated with anti-μ antibodies (Fig. 1). In BL cells, however, pretreatment with anti-δ only marginally affected a subsequent anti-μ response (Fig. 1). This result probably results from the fact that, as stated above, anti-δ antibodies induce a calcium response in only 40% of the cells.

We subsequently analyzed the ability of anti-immunoglobulin antibodies to desensitize normal human B cells. T-cell-depleted mononuclear cell preparations were used as a source of blood B cells. The number of B cells [as determined by staining with anti-immunoglobulin reagents and pan-B cell monoclonal antibodies (CD20)] varied between 30 and 50% in individual preparations. Incubation of these cell preparations with either anti-μ or anti-δ antibodies increases $[Ca^{2+}]_i$ in a percentage of cells equal to the percentage of B cells in the population (Fig. 1 *C* and *D*). Because the anti-μ-induced calcium response in T-cell-depleted mononuclear cell preparations is confined to $CD20^{+ve}$ B cells (G.T.R., unpublished result), it can be concluded that both anti-μ and anti-δ antibodies can induce Ca^{2+} mobilization in the vast majority of, if not all, blood B cells.

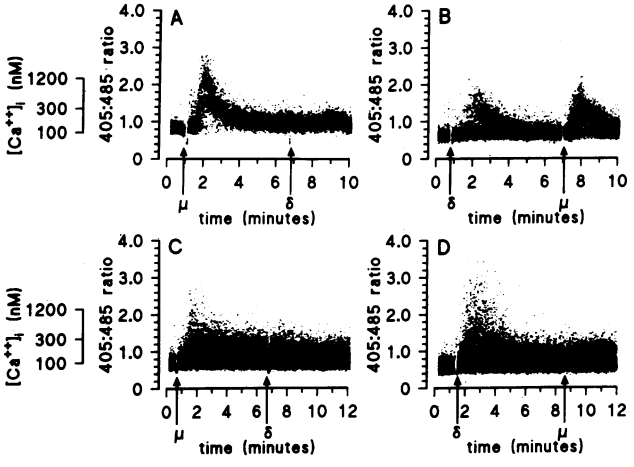

FIG. 1. Anti-immunoglobulin-induced desensitization in the B-cell line BLgpt1 (*A* and *B*) and normal human B cells (*C* and *D*). (*A* and *C*) Indo-1-loaded BLgpt1 B cells and normal B cells were analyzed for $[Ca^{2+}]_i$ by flow cytometry before and after stimulation with $F(ab')_2$ fragments of goat anti-human IgM (μ) at 1 μg/ml, followed by stimulation with goat anti-human IgD (δ) at 1 μg/ml to assess desensitization. (*B* and *D*) Cells were first stimulated with anti-δ and then by anti-μ. In dot-plot format are shown the changes in 405/485-nm indo-1 fluorescence ratio over time. Corresponding $[Ca^{2+}]_i$ (in nM) is indicated. Data represent five independent experiments.

We next investigated whether peripheral blood B cells could be rendered unresponsive to anti-δ stimulation by pretreatment with anti-μ and vice versa. B cells were activated with optimal doses of anti-μ, and $[Ca^{2+}]_i$ was monitored. When $[Ca^{2+}]_i$ had returned to near-baseline values, cells were stimulated with anti-δ in optimal concentrations. Data shown in Fig. 1*C* indicate that this particular pretreatment regimen renders blood B cells completely unresponsive to anti-δ activation. Furthermore, we also observed that pretreatment of blood cells with optimal stimulatory doses of anti-δ renders them unresponsive to a subsequent anti-μ challenge (Fig. 1*D*). These data indicate that in Epstein–Barr virus-transformed human B cells, as well as in fresh blood B cells, triggering of surface immunoglobulin molecules with polyclonal or monoclonal reagents leads to desensitization of unoccupied receptor because ligation of these surface immunoglobulin molecules does not induce a rise in $[Ca^{2+}]_i$.

Anti-μ Induces Desensitization of CD19, CD21, and Bgp95. The finding that treatment of human B cells with anti-μ renders the cells unresponsive to subsequent activation with anti-δ raises the possibility that other receptors, which are linked to the PLC second-messenger system are also desensitized. To address this possibility, peripheral blood B cells were treated with optimal doses of goat anti-human IgM. After $[Ca^{2+}]_i$ had returned to near-baseline values, CD19 (0.1 μg/ml), CD21 (0.1 μg/ml), or anti-Bgp95 (20 μg/ml) was added, followed after 5 min by addition of cross-linking goat anti-mouse immunoglobulin. Anti-μ pretreatment completely abolished any increase in $[Ca^{2+}]_i$, which otherwise is seen after crosslinking of CD19, CD21, or Bgp95 (Fig. 2), suggesting that the phenomenon of ligand-induced mIg desensitization is not restricted to membrane immunoglobulin molecules but apparently involves other Ca^{2+} mobilization-coupled membrane determinants.

Effect of Desensitizing Anti-μ Treatment on Expression of mIgD, CD19, CD21, and Bgp95. The observation that treatment with anti-μ renders B cells unresponsive to stimuli that normally mobilize Ca^{2+} could be explained by an anti-μ-induced alteration in accessibility of the respective transducer molecules to ligand. To address this hypothesis, we compared the surface IgD, CD19, CD21, and Bgp95 staining pattern of blood B cells that had or had not been pretreated with anti-μ antibodies. Anti-μ treatment did not influence staining pattern of surface IgD, CD19, CD20, CD21, or Bgp95, either in percentage of staining cells or mean fluo-

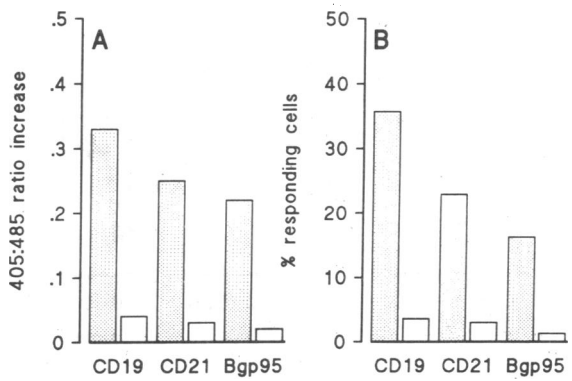

FIG. 2. Indo-1-loaded peripheral blood B cells were incubated for 30 min at 37°C with anti-μ (open bars) at 1 μg/ml or medium (shaded bars). Subsequently, cells were stimulated with anti-CD19, -CD21, or -Bgp95, and then crosslinked with goat anti-mouse immunoglobulin. Data are presented as maximal increase in $[Ca^{2+}]_i$ seen (*A*) and as percentage responding cells (*B*). Data represent four replicate experiments.

rescence intensity of staining cells (data not shown). Because our conventional surface staining protocol is based on a 30-min incubation on ice with primary monoclonal antibody, it cannot be excluded on these data that anti-μ treatment influences the kinetics of binding of the monoclonal antibody to the respective cell-surface determinant. Therefore, we analyzed the kinetics of binding of the respective antibodies. Cells were incubated for 0, 2.5, 5, 10, or 20 min with anti-IgD, CD19, CD20, CD21, or Bgp95, washed, and incubated for 30 min with fluoresceinated goat anti-mouse immunoglobulin. Subsequent analysis showed that anti-μ treatment did not affect antibody binding to IgD, CD19, CD20, CD21, or Bgp95 (Fig. 3). It, thus, can be concluded that anti-μ-induced desensitization does not result from receptor down-regulation, modulation, steric hindrance, or other phenomena that would decrease accessibility of cell-surface determinants to respective ligands.

Dose Dependence and Longevity of Desensitization. The experiments described above were done with optimal doses of anti-μ antibodies—i.e., 1 μg of F(ab')$_2$ fragments of affinity-purified goat anti-human IgM per ml. Experiments in which 5-fold, 25-fold, or 125-fold lower concentrations were used clearly showed the dose dependency of the anti-μ-induced calcium response. At lower concentrations of anti-μ, the time required to reach maximal $[Ca^{2+}]_i$ is prolonged from 30 sec at 1 μg/ml to 150 sec at 0.008 μg/ml. Similarly, lower doses of anti-μ induce a Ca^{2+} response of lower magnitude; maximal relative $[Ca^{2+}]_i$ obtained with 1 μg/ml is 0.60, whereas with 0.008 μg/ml, this is only 0.28 (Fig. 4). The ability of anti-δ antibodies and anti-CD21 antibodies, used in an optimal concentration of 1 μg/ml, to increase $[Ca^{2+}]_i$ in B cells pretreated with these various doses of anti-μ was investigated. In B cells treated with low doses of anti-μ (0.008–0.04 μg/ml), anti-δ antibodies induced an increase in $[Ca^{2+}]_i$, which in magnitude is ≈30% of that obtained in control B cells (Fig. 4). Crosslinking of CD21 on B cells previously treated with low doses of anti-μ only marginally increased $[Ca^{2+}]_i$; this increase was 10–15% that in control B cells. These data indicate that maximal desensitization is induced only when the desensitizing agent itself induces optimal Ca^{2+} mobilization. Furthermore, nonimmunoglobulin determinant-triggered calcium responses are at least as susceptible to anti-μ-induced desensitization as anti-δ-induced responses.

Anti-μ-induced desensitization occurs within the time required for $[Ca^{2+}]_i$ to return to near baseline after stimulation (see Fig. 1). We have performed a series of experiments to investigate the duration of this state of unresponsiveness to anti-δ, anti-CD19. Indo-1-loaded B cells were incubated at

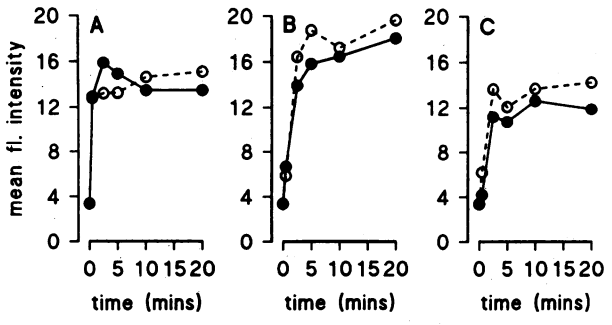

FIG. 3. Desensitization and receptor expression. Peripheral blood B cells were treated with medium (○) or with anti-μ at 1 μg/ml (●) for 30 min at 37°C. Cells were washed and incubated for indicated times with anti-IgD (*A*), anti-CD19 (*B*), or anti-CD21 (*C*). Next, cells were washed and incubated with fluoresceinated goat anti-mouse immunoglobulin followed by an incubation with phycoerythrin-conjugated anti-CD20 (Leu 16). Mean green fluorescence intensity of CD20^{+ve} cells is plotted versus time.

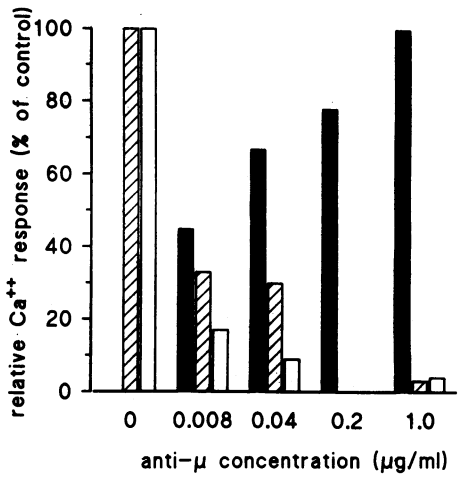

FIG. 4. Dose dependence of anti-μ-induced B-cell desensitization. Indo-1-loaded peripheral blood B cells were stimulated with variable doses of anti-μ (closed bars). After $[Ca^{2+}]_i$ had returned to near-baseline values, cells were stimulated with anti-δ at 1 μg/ml (hatched bars) or anti-CD21 at 0.1 μg/ml followed by goat anti-mouse immunoglobulin at 0.5 μg/ml (open bars). The $[Ca^{2+}]_i$ response, relative to that obtained in nonpretreated cells, is graphed.

37°C with anti-μ at 1 μg/ml and at various times thereafter (to 4 hr) activated with anti-δ or anti-CD19, and $[Ca^{2+}]_i$ was monitored. Alternatively, B cells were incubated with anti-μ for 45 min, washed, and incubated in fresh RPMI 1640 medium at 37°C until analysis. Cells were then loaded with indo-1 and activated with anti-δ or anti-CD19. In all cases, the calcium response of B cells pretreated only with medium served as a control. Data summarized in Fig. 5 indicate that before 16 hr after activation with anti-μ, the anti-δ response still is severely impaired. It should be noted, however, that at 16 hr a small, but significant, percentage (≈20%) of B cells does show an increase in $[Ca^{2+}]_i$, which is comparable in magnitude with untreated B cells. At this time, crosslinking of CD19 still failed to induce any significant increase in $[Ca^{2+}]_i$ (Fig. 5).

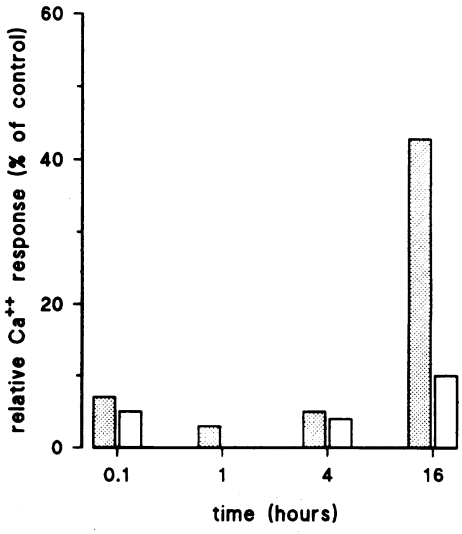

FIG. 5. Longevity of B-cell desensitization. Indo-1-loaded peripheral blood B cells were stimulated with anti-μ at 1 μg/ml and kept at 37°C. Five minutes, 1 hr, or 4 hr later B cells were stimulated with anti-δ (shaded bars) or anti-CD19 (open bars). Alternatively, B cells were activated with anti-μ for 45 min, washed, and incubated at 37°C for 15 hr. Next, B cells were loaded with indo-1 and stimulated with anti-δ or anti-CD19. The Ca^{2+} response is expressed as percentage of the response seen in B cells not treated with anti-μ.

Anti-μ-Induced Desensitization Does Not Impair Induction of Ca^{2+} Mobilization by G Protein-Activating Agents or Ca^{2+} Ionophores. Anti-immunoglobulin stimulation could potentially induce desensitization by affecting any intermediary step between receptor ligation and Ca^{2+} release into the cytosol. To find the site of action of desensitization, we conducted experiments in which cells were stimulated after anti-μ desensitization by agents that induce Ca^{2+} mobilization by targeting G proteins or Ca^{2+} stores directly. First we studied whether treatment of blood B cells with anti-μ impairs the capacity of the calcium ionophore ionomycin to saturate intracellular trapped indo-1 with calcium. We found that in both untreated and anti-μ-treated B cells, 1 μM ionomycin induces a massive influx of extracellular calcium, thereby saturating intracellular indo-1 (causing 3.83- and 4.37-fold increases in indo-1 ratios, respectively). We next addressed the possibility that anti-μ-induced desensitization is mediated by inactivation of some event at or downstream from G protein activation by assessing ability of the G protein-activating agent AlF_4^- (18) to trigger Ca^{2+} mobilization in anti-μ-treated and control B cells. Data in Fig. 6 indicate that anti-μ treatment does not impair the ability of AlF_4^- to increase $[Ca^{2+}]_i$. In both untreated and anti-μ-treated cells, AlF_4^- increases $[Ca^{2+}]_i$ in all cells. In anti-μ-treated cells this response was more rapid, and maximal $[Ca^{2+}]_i$ attained was higher than in nondesensitized cells. Thus, anti-μ-induced desensitization does not negatively affect the ability of a B cell to mobilize intracellular calcium when the sequence of intracellular events originates at or downstream from the PLC-regulating G protein.

DISCUSSION

The data presented in this paper show that crosslinking of surface IgM on human B cells with anti-μ heavy chain reagents induces a state of unresponsiveness in which occupancy of normally calcium-coupled immunoglobulin, CD19, CD21, and Bgp95 does not result in an increase in $[Ca^{2+}]_i$. These data confirm and extend earlier observations made using murine B cells (refs. 8, 9, 18). In those studies it was shown that occupancy of 2–10% of surface IgD molecules on quiescent splenic B cells renders these cells unresponsive to subsequent stimulation with the same (anti-δ) or reciprocal (anti-μ) antibody. Anti-μ antibodies also induced desensitization of unligated receptors. This desensitization does not involve protein kinase C as a primary mechanism and appears to reflect uncoupling of membrane immunoglobulin from G proteins (1–3, 18). In previous studies, it was not possible to determine whether anti-immunoglobulin induced desensitization affected the ability of other B-cell receptors to transduce signals because in the mouse, no other calcium mobilization-coupled surface markers have been described. The data described here advance previous knowledge by demonstrating ligand-induced desensitization is not limited to membrane immunoglobulin but affects other calcium-mobilizing ability of other receptors, including CD19, CD21, and Bgp95.

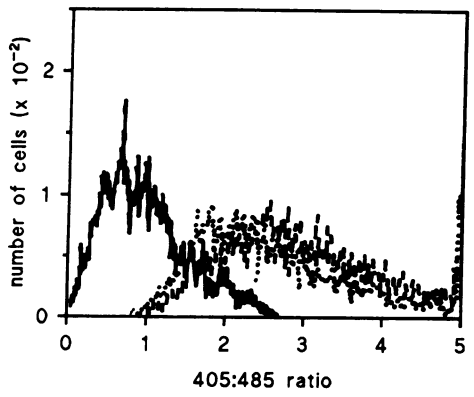

FIG. 6. Anti-μ-induced desensitization does not affect responsiveness to AlF_4^-. Indo-1-loaded peripheral blood B cells were stimulated with anti-μ (- -) at 1 μg/ml for 30 min at 37°C or with medium (•••). Subsequently, cells were stimulated with AlF_4^- (10 μM $AlCl_3$/30 mM NaF). Relative $[Ca^{2+}]_i$, expressed as 405/485 ratio, was determined before (—) and 30 min after AlF_4^- addition.

In human B cells, three non-immunoglobuli determinants are known that apparently are linked to the PLC second-messenger system because their crosslinking by monoclonal antibodies leads to an increase in $[Ca^{2+}]_i$. Of these three molecules, only CD21 is a known receptor, being CR2, the receptor for CD3 (11). Experiments in which CR2 is ligated either with CD3 or with CD21 monoclonal antibodies are suggestive for a role of C3d (28) as a B-cell growth and/or differentiation factor (19–21). The natural ligands for CD19 and Bgp95 are unknown. The fact that the binding of a monoclonal antibody to CD19 and Bgp95 induces calcium mobilization suggests that these molecules have a receptor function. Furthermore, antibodies to Bgp95 used alone or in combination with anti-μ, induce B-cell proliferation (14). Under appropriate conditions, anti-CD19 antibodies can inhibit anti-μ-induced B-cell proliferation (22). Recent evidence indicates that CD19 and membrane IgM may be associated on the cell surface (23). Therefore, the natural ligands for CD19 and Bgp95 may play important roles in physiologic regulation of B-cell activation and differentiation. The data presented in this paper suggest a mechanism for regulation of the receptor function by means of receptor crosstalk. For example, when the immune response of a B cell is triggered by antigen binding, one of the consequences of that binding will be a period of refractility to ligands of CD19, CD21, or Bgp95. This period during which some B-cell receptors are inactive may provide an important temporary state of rest for the cell during which the cell can process and act on previously received information by, for example, moving to a new differentiative stage.

Antigen receptor desensitization, provided it is maintained for a long period, could also be manifest as B-cell tolerance. In a recently described transgenic mouse model, in which all B cells express surface IgM and IgD with a specificity for hen egg lysozyme, it was demonstrated that *in vivo* exposure to hen egg lysozyme renders all B cells (both immature and mature) immunogen unresponsive (10, 11, 24). The data presented in this paper as well as the data from Cambier *et al.* (8, 19) and Harnett *et al.* (9) could provide the molecular mechanism for clonal anergy, the condition in which antigen stimulation of mature B cells renders them unresponsive to subsequent stimulation with an immunogenic form of the same antigen.

In most other systems thus far studied (for review, see ref. 25), receptor desensitization appears to be mediated by receptor phosphorylation. However, phosphorylative inactivation of mIg is impossible because these receptors do not possess phosphate acceptor sites in their cytoplasmic tails. In murine B cells, mIg desensitization may be explained in molecular terms by the existence of mIg associated proteins that act as transducers or regulators (26, 27). These, as yet poorly defined, molecules are tyrosine phosphorylated upon B-cell stimulation by anti-immunoglobulin and are believed to constitute the link between immunoglobulin receptor and G protein (refs. 27 and 28, K. Campbell and J.C.C., unpublished work). In this model, anti-μ- or anti-δ-induced phosphorylation of these transducer molecules would lead to desensitization of both membrane IgM and IgD receptor complexes. CD19, CD21, and Bgp95 or their transducers could also be substrates of the operative kinase.

The skillful technical and secretarial assistance of Mrs. E. J. Scholtens and also the secretarial assistance of Mrs. L. Gramberg

and A. van Dooren are gratefully acknowledged. G.T.R. was supported, in part, by a grant from the Netherlands Organization for Scientific Research (NWO Grant R983-174.89). J.C.C. is the Cecil and Ida Green Professor of Cell Biology.

1. Gold, M. R., Jakway, J. P. & DeFranco, A. L. (1987) *J. Immunol.* **139,** 3604–3613.
2. Harnett, M. M. & Klaus, G. G. B. (1988) *J. Immunol.* **140,** 3135–3139.
3. Monroe, J. & Haldar, S. (1989) *Biochim. Biophys. Acta* **1013,** 273–278.
4. Cambier, J. C., Justement, L. B., Newell, M. K., Chen, Z. Z., Harris, L. K., Sandoval, V. M., Klemsz, M. J. & Ransom, J. T. (1987) *Immunol. Rev.* **95,** 37–59.
5. Imboden, J. & Pattison, G. (1987) *J. Clin. Invest.* **79,** 1538–1541.
6. Nishizuka, Y. (1986) *Science* **233,** 305–312.
7. Cambier, J. C. & Ransom, J. T. (1987) *Annu. Rev. Immunol.* **5,** 175–199.
8. Cambier, J. C., Chen, Z. Z., Pasternak, J., Ransom, J. T., Sandoval, V. & Pickles, H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6493–6497.
9. Harnett, M. M., Holman, M. J. & Klaus, G. G. B. (1989) *Eur. J. Immunol.* **19,** 1935–1939.
10. Pike, B. L., Boyd, A. W. & Nossal, G. J. V. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2013–2017.
11. Goodnow, C. C., Crosbie, J., Adelstein, S., Lavoie, T. B., Smith-Gill, S. J., Brink, R. A., Pritchard-Briscoe, H., Witherspoon, J. S., Loblay, R. H., Raphael, K., Trent, R. J. & Basten, A. (1989) *Nature (London)* **334,** 676–682.
12. Uckun, F. M., Jaszcz, W., Ambrus, J. L., Fauci, A. S., Gajl-Peczalska, K., Song, C. W., Wick, M. R., Myers, D. E., Waddick, K. & Ledbetter, J. A. (1988) *Blood* **71,** 13–29.
13. Carter, R. H., Spycher, M. O., Ng, Y. C., Hoffman, R. & Fearon, D. T. (1988) *J. Immunol.* **141,** 457–463.
14. Valentine, M. A., Clark, E. A., Shu, G. L., Norris, N. A. & Ledbetter, J. A. (1988) *J. Immunol.* **140,** 4071–4078.
15. Griffioen, A. W., Rijkers, G. T. & Zegers, B. J. M. (1989) *J. Immunol. Methods* **120,** 23–27.
16. Keij, J. F., Griffioen, A. W., The, T. H. & Rijkers, G. T. (1989) *Cytometry* **6,** 814–819.
17. Gregory, C. D., Tursz, T., Edwards, C. F., Tetaud, C., Talbot, M., Caillou, B., Rickinson, A. & Lipinski, M. (1987) *J. Immunol.* **139,** 313–318.
18. Cambier, J. C., Fisher, C. L., Pickles, H. & Morrison, D. C. (1990) *J. Immunol.* **145,** 13–19.
19. Nemmerow, G. R., McNaughton, M. E. & Cooper, N. R. (1985) *J. Immunol.* **135,** 3068–3073.
20. Melchers, F., Erdei, A., Schulz, T. & Dierich, M. P. (1985) *Nature (London)* **266,** 264–266.
21. Bohnsack, J. F. & Cooper, N. R. (1988) *J. Immunol.* **141,** 2569–2576.
22. Rabinovitch, P. S., Clark, E. A., Pezzutto, A., Ledbetter, J. A. & Draves, K. E. (1987) in *Leukocyte Typing III*, ed. McMichael, A. J. (Oxford Univ. Press, Oxford), pp. 349–352.
23. Pesando, J. M., Bouchard, L. S. & McMaster, B. (1990) *J. Exp. Med.* **170,** 2156–2164.
24. Goodnow, C. C., Crosbie, J., Jorgensen, H., Brink, R. A. & Basten, A. (1989) *Nature (London)* **342,** 385–391.
25. Benovic, J. L., Bourier, M., Caron, M. G. & Lefkowitz, R. G. (1990) *Annu. Rev. Cell. Biol.* **4,** 405–428.
26. Campbell, K. S. & Cambier, J. C. (1990) *EMBO J.* **9,** 441–448.
27. Weinands, J., Hombach, J., Radbruch, A., Riesterer, C. & Reth, M. (1990) *EMBO J.* **9,** 449–456.
28. Ross, G. D., Newman, J. D., Lambris, J. D., Davery-Pocius, J. E., Cain, J. A. & Lechmann, P. J. (1983) *J. Exp. Med.* **158,** 334–342.