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Ligand-induced desensitization of B-cell membrane immunoglobulin-mediated Ca^{2+} mobilization and protein kinase C translocation

(IgM/IgD/transmembrane signaling/antigen receptor)

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ABSTRACT **Binding of ligand to B-cell membrane immunoglobulin (mIg) can lead to activation of a number of distinct biologic responses, including altered expression of genes encoding c-fos, c-myc, and Ia, as well as proliferation and immunologic tolerance. Tolerance could reflect a functional uncoupling of receptors from systems that generate intracellular second messengers (i.e., receptor desensitization). To better understand the molecular basis of immune regulation, we examined the ability of mIg to function as a signal transducer after the cell's initial contact with mIg-binding ligand. The results show that ligand binding to as little as 2–10% of mIgM or mIgD renders the cell unresponsive to ligand binding to the reciprocal isotype as judged by Ca^{2+} mobilization and protein kinase C translocation responses. This heterologous receptor desensitization lasts longer than 24 hr and does not reflect loss of receptor from the cell surface. Studies with the calcium ionophore ionomycin, 1,2-dioctanoyl-*sn*-glycerol, and the protein kinase inhibitor staurosporine indicate that both protein kinase C-dependent and protein kinase C-independent (staurosporine-insensitive) mechanisms mediate heterologous desensitization after mIg crosslinking.**

Resting peripheral B lymphocytes coexpress antigen receptors of two immunoglobulin isotypes, membrane IgM (mIgM) and mIgD (1, 2). Ligand binding to mIgM and/or mIgD results in transmembrane signal transduction resulting in altered gene transcription leading to cell activation (M. J. Klemsz, E. Palmer, and J.C., unpublished data; refs. 3–5). It is not known whether receptor mIgM and mIgD function at all times or whether their ability to transduce signals is regulated analogously to many other cell surface receptors, most notably adrenergic receptors, which are inactivated or desensitized by a phosphorylation event (for review see ref. 6). Consistent with the possibility that the function of these receptors is actively regulated are observations that (*i*) "natural" B blasts isolated from mouse spleens are unresponsive to mitogenic anti-IgM antibodies despite the fact that they express normal amounts of mIgM (7), (*ii*) under some situations interaction of B cells with antigen renders them "tolerant" or unresponsive to a later challenge with the same antigen in immunogenic form (for review see ref. 8), and (*iii*) phorbol diesters, which activate protein kinase C (PKC), induce B cells to become unresponsive to subsequent anti-Ig stimulation (9, 10). Thus, ligand binding to receptor mIg may render that receptor, and perhaps neighboring receptors of the same type, unable to transduce signals following a subsequent ligand binding event.

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Seemingly at odds with the concept of ligand-mediated desensitization of mIg is the fact that antigen (11) or surrogate anti-Ig antibodies (12) must be present throughout the culture period to induce an optimal *in vitro* B-cell immune response. If receptor desensitization occurs and is of long duration, one would expect that ligand should only be required early during stimulation.

Until recently it has been impossible to determine whether antigen responsiveness is regulated at the level of the antigen receptor because the basis of signal transduction by mIg was not defined sufficiently to provide appropriately early reporters of signal transduction. However, it is now evident that mIg-mediated signaling leading to altered gene expression is mediated by hydrolysis of inositolphospholipids by a phospholipase C leading to generation of inositol phosphate and diacylglycerol second-messenger molecules (for review see ref. 13). These messengers mediate mobilization of intracellular Ca^{2+} (14, 15), extracellular Ca^{2+} influx (16, 17), and translocation (18) and activation (19) of PKC.

We reasoned that if ligation of some proportion of mIg leads to inactivation of the cell's remaining antigen receptors, a phenomenon referred to as heterologous receptor desensitization, the effect should be reflected by a loss of the ability to evoke the normal Ca^{2+} mobilization and PKC translocation responses upon ligand binding to the remaining unoccupied mIg. This problem can be addressed most easily by assessing the effect of anti-μ antibodies on subsequent responsiveness to anti-δ antibodies and vice versa. In this way, possible ambiguities caused by ligand competition for receptors are eliminated.

Here we describe studies that indicate that ligand binding to mIgM or mIgD for as little as 3 min renders the cell unable to respond to subsequent ligand binding to the reciprocal receptor by undergoing Ca^{2+} mobilization or PKC translocation responses. Ligation of only 2–10% of mIg results in desensitization. This desensitization is long-lived (>24 hr), does not reflect modulation of the mIg, and appears not to be mediated solely by PKC.

MATERIALS AND METHODS

Animals and Reagents. BDF_1 mice produced in the National Jewish Center animal facility were used at an age of 8–12 weeks. Monoclonal antibodies used included JA12.5, a rat

Abbreviations: mIg, membrane immunoglobulin; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; PKC, protein kinase C; diC_8, 1,2-dioctanoyl-*sn*-glycerol.

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anti-mouse δ chain (IgD heavy chain) obtained from Joe Davie (Washington University, Saint Louis); M1.42.3.9.8, a rat anti-H-2K (20) obtained from John Kappler (National Jewish Center, Denver); b-7-6, a rat anti-mouse μ chain (IgM heavy chain) (21) obtained from C. Heusser (CIBA–Geigy, Basel); and D3.137, a mouse anti-I-A^{db} obtained from Sue Tonkonogy (University of North Carolina School of Veterinary Medicine, Raleigh, NC). Indo-1 pentakis(acetoxymethyl ester) (AM) was purchased from Molecular Probes (Eugene, OR). 1,2-Dioctanoyl-*sn*-glycerol (diC_8) was purchased from Avanti Polar Lipids and ionomycin and staurosporine were from Calbiochem.

B-Cell Preparation. Spleens were excised from mice killed by CO_2 asphyxiation, and cells were dispersed by being forced through 110-μm polyethylene mesh (Spectrum Medical Industries, Los Angeles). Cells were washed and depleted of erythrocytes by use of Gey's solution. T cells were then depleted by incubation with a mixture of anti-Thy-1 antibodies (T24/30, HO-134) and rabbit complement. Resting B cells were further purified by sedimentation through Percoll (Pharmacia) as described (22). Those cells with density >1.085 (20–30% of total B cells) were used in the studies described here. Greater than 95% of these cells were in G_0 as judged by cytofluorimetric analysis of acridine orange-stained cells (data not shown).

Analysis of Intracellular Free Ca^{2+} Concentration ($[Ca^{2+}]_i$). Small B cells were washed and suspended at 7.5 × 10^6 per ml in warm Hanks' balanced salts solution (BSS, pH 7.0) containing 5 μM indo-1 AM. After incubation for 30 min at 37°C to effect loading, cells were diluted 1:1 with BSS (pH 7.4) containing 5% (vol/vol) fetal bovine serum (HyClone, Logan, UT) and incubated an additional 30 min at 37°C. Cells were then washed and resuspended in BSS containing 5% fetal bovine serum and maintained at 4°C until being warmed to 37°C 30 min before analysis. $[Ca^{2+}]_i$ cells loaded with indo-1 (23) was determined by flow microfluorimetry (Cytofluorograf 50H, Ortho Diagnostics) as described (24). Analyses were conducted at a sample temperature of 37°C and a flow rate of 600–700 cells per sec. Data are presented as isometrics displaying $[Ca^{2+}]_i$ as a function of time and cell number. In some figures, the mean $[Ca^{2+}]_i$ of the cell population is plotted against time.

Analysis of PKC Localization. PKC levels in subcellular fractions were monitored based upon binding of [^{3}H]phorbol 12,13-dibutyrate binding. Small B cells were suspended in BSS containing 5% fetal bovine serum at 37°C (10^7 cells per 1-ml sample) and stimulated for various periods before being cooled rapidly, washed, disrupted, fractionated, and assayed for PKC as described (25, 26).

Immunofluorescence Analysis. Surface immunoglobulin expression was determined by continuous flow microfluorimetric analysis (Cytofluorograf 50H with appended 2150 computer system) of cells (600–700 cells per sec) after mixture with fluoresceinated avidin and before and after addition of biotinylated monoclonal anti-δ (JA12.5) or anti-μ (b-7-6) antibodies. In isometric displays, immunofluorescence (3-decade logarithmic scale) is expressed as a function of time and relative cell number. Nonviable cells were eliminated from the analysis by gating based on forward light scatter.

RESULTS

Anti-IgM and Anti-IgD Induction of Ca^{2+} Mobilization and Heterologous Receptor Desensitization. We first assessed the ability of small B cells to respond to sequential stimulation with monoclonal anti-μ (b-7-6) and anti-δ (JA12.5) antibodies, and vice versa, by mobilization of intracellular Ca^{2+}. We chose to use antibodies against one isotype (e.g., anti-μ) followed by antibodies against the other (e.g., anti-δ) to exclude potential ambiguities caused by antibody competition for the same receptor. It should be noted that virtually all small B cells express both mIgM and mIgD (1). Isometric displays of data generated by flow microfluorimetric analysis of indo-1-loaded cells (Fig. 1) illustrate changes in $[Ca^{2+}]_i$ that follow stimulation of small B cells with anti-μ (b-7-6), anti-δ (JA12.5), or anti-Ia (D3.137) at 10 μg/ml. Stimulation of cells with anti-δ led to an increase in $[Ca^{2+}]_i$ in virtually all cells; in some cases, $[Ca^{2+}]_i$ exceeded 700 nM. A similar response was seen after stimulation with anti-μ, although the maximal $[Ca^{2+}]_i$ observed was somewhat lower. Antibody binding to Ia did not lead to Ca^{2+} mobilization. Anti-Ia stimulation did not adversely affect the cells' ability to respond to subsequent stimulation by anti-δ. However, cells stimulated with anti-δ mobilized Ca^{2+} very poorly in response to a subsequent exposure to anti-μ and vice versa. Thus, ligand binding to mIgM or mIgD renders cells unable to respond normally to subsequent crosslinking of the reciprocal receptor as indicated by Ca^{2+} mobilization. This effect appears to be independent of potential Fc receptor-mediated effects, since whole molecules and $F(ab')_2$ fragments of anti-δ antibodies (JA12.5) were equally effective inducers of desensitization when used at 10 μg/ml per 10^6 cells. Further, the monoclonal anti-Fc receptor antibody 2.4G2 (27) did not block induction of desensitization (data not shown).

Anti-IgM and Anti-IgD Induce Heterologous Receptor Desensitization as Indicated by PKC Translocation. Crosslinking of mIg on B cells normally leads to activation of a bifurcating transduction cascade involving two second messengers, Ca^{2+} and diacylglycerol. Diacylglycerol generated after mIg crosslinking mediates translocation of PKC from the cytosol to the plasma membrane (13), where it is bound and becomes

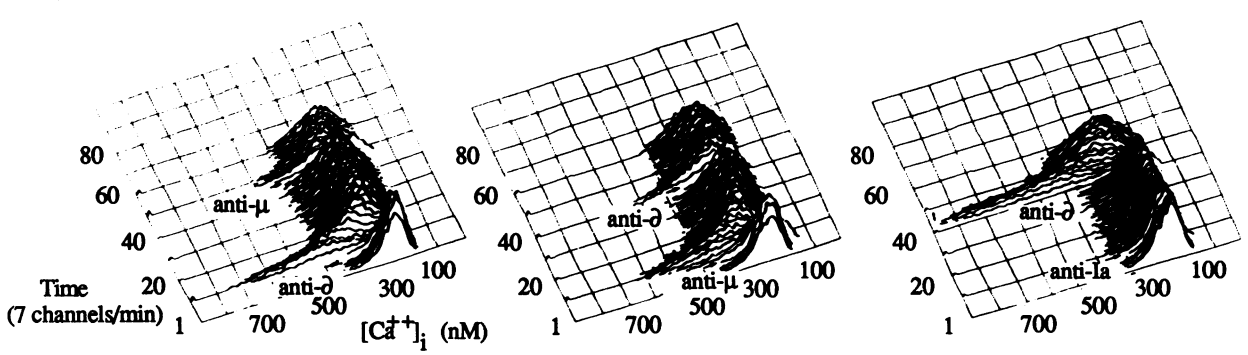

FIG. 1. Anti-IgM and anti-IgD antibodies induce heterologous desensitization of mIg as measured by Ca^{2+} mobilization. Isometric displays of data generated by flow cytometry of indo-1-loaded cells. Indo-1-loaded small B cells (2 × 10^6 per ml) were analyzed (700–800 cells per sec) to establish basal $[Ca^{2+}]_i$ before stimulation as indicated with monoclonal anti-δ (JA12.5), anti-μ (b-7-6), or anti-Ia (D3.137) antibodies (10 μg/ml). Data are representative of >10 replicate experiments.

activated by virtue of an association with a complex of diacylglycerol, phosphatidylserine, and Ca^{2+} (18). To determine whether desensitization of mIg involves the diacylglycerol as well as the Ca^{2+} arm of the signaling cascade, we assessed whether pretreatment of B cells with anti-μ or anti-δ renders them unresponsive to a subsequent challenge with the reciprocal antibody by undergoing a PKC translocation response. Stimulation of B cells with anti-μ (b-7-6) or anti-δ (JA12.5) at 10 μg/ml leads to translocation of ≈80% of phorbol ester-binding activity, previously shown to reflect PKC (25, 26), from the cytosolic compartment to the Triton X-100-soluble membrane compartment (Fig. 2). The original compartmentation was restored within 60 min. Stimulation of cells with the reciprocal antibody (10 μg/ml) 60 min after prestimulation did not lead to a second PKC translocation response. Thus, ligand binding to mIgM or mIgD leads to desensitization of both Ca^{2+} mobilization and PKC translocation responses after ligation of the reciprocal antigen receptors.

mIg Desensitization Does Not Reflect Receptor Modulation. A possible explanation for the observed findings is that mIgM (mIgD) crosslinking in some way causes modulation, by endocytosis or shedding, of mIgD (mIgM). To address this possibility, cells were treated for 5 min with desensitizing doses of anti-μ or anti-δ before analysis of binding of ligand to the reciprocal immunoglobulin. Control cell populations containing fluoresceinated avidin only or cell suspensions containing desensitizing antibody plus fluoresceinated avidin were analyzed continuously by flow microfluorimetry to establish the background fluorescence. Analysis was stopped briefly while biotinylated antibody was added, and then the analysis was continued. Pretreatment of cells with anti-μ antibodies (10 μg/ml) did not affect either the rate or the maximal level of binding of fluorescent avidin and the biotin-anti-δ complex to B cells (Fig. 3). The reciprocal analysis revealed that anti-δ (10 μg/ml) did not affect binding of fluorescent avidin and biotin-anti-μ to mIgM (data not shown). Thus, the heterologous receptor desensitization observed is not mediated by loss of mIgM or mIgD from the cell surface. The relevant receptor is present but is uncoupled from second-messenger systems that mediate the Ca^{2+} mobilization and PKC translocation.

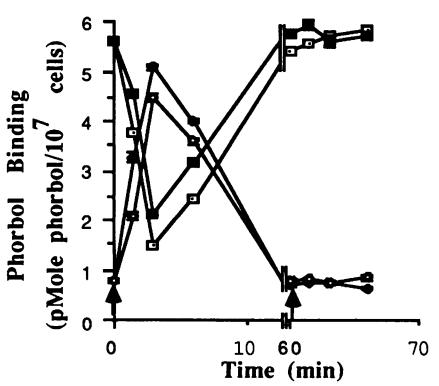

FIG. 2. Anti-IgM and anti-IgD antibodies induce heterologous desensitization of mIg as indicated by PKC translocation. Small B cells (2 × 10^7 per ml) were stimulated with anti-μ (b-7-6, 10 μg/ml, solid symbols) or anti-δ (JA12.5, 10 μg/ml, open symbols) antibodies for 0–10 min before 10^7 cells were removed, and membrane (◇, ◆) and cytosolic (□, ■) fractions were prepared and analyzed for PKC translocation based on [^{3}H]phorbol dibutyrate binding. The remaining cells were cultured 60 min before addition of the reciprocal antibody (10 μg/ml). After incubation for 0–10 min cultures were harvested and PKC localization was assessed. Mean and standard deviation of three replicate assays are shown. Data are representative of three replicate experiments.

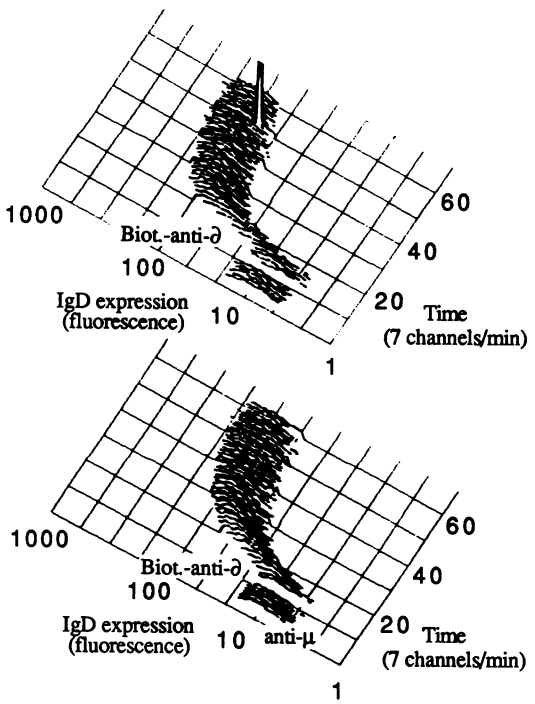

FIG. 3. mIg desensitization does not reflect a decrease of mIg on the cell surface. Suspensions of small B cells (10^6 per ml) containing fluoresceinated avidin were stimulated with a desensitizing dose of anti-μ (b-7-6, 10 μg/ml) (*Lower*) or cultured without stimulus (*Upper*) for 5 min before analysis for IgD expression by continuous monitoring of cell-associated fluorescence after addition of biotin-conjugated anti-δ (JA12.5). Isometric displays illustrate cell staining as a function of time. Data are representative of four replicate experiments.

Antibody-Dose Requirements for Induction of Desensitization. We next analyzed ligand-dose requirements for induction of desensitization. Indo-1-loaded cells were stimulated with various concentrations of anti-μ or anti-δ, and $[Ca^{2+}]_i$ was analyzed for 5 min before addition of the reciprocal antibody (10 μg/ml) and continuation of analysis for 10 min. Maximum mean $[Ca^{2+}]_i$ responses to the second stimulus were recorded and were plotted (Fig. 4) as a percentage of the response of control cells, which were not prestimulated. Prestimulation with ≥0.1 μg of anti-δ (JA12.5) resulted in significant desensitization (≈50%) of the response to anti-μ. We estimate from immunofluorescence staining analysis

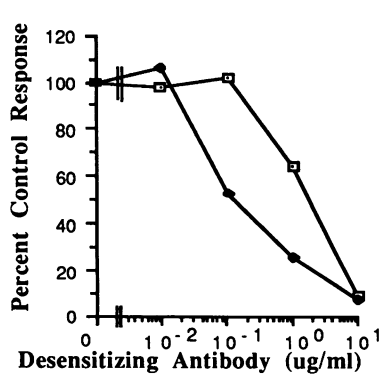

FIG. 4. Ligand-dose dependence for induction of desensitization. Indo-1-loaded small B cells (2 × 10^6 per ml) were cultured with various amounts of anti-μ (b-7-6, ◆) or anti-δ (JA12.5, □) antibodies for 5 min before addition of anti-δ (◆) or anti-μ (□) at 10 μg/ml and analysis of $[Ca^{2+}]_i$ by flow cytometry. Desensitization was measured as the reduction of the mean maximum increase in $[Ca^{2+}]_i$ that followed exposure to the second antibody. Data are representative of three replicate experiments.

(data not shown) that 0.1 μg of anti-δ per 10^6 cells per ml is sufficient to saturate ≈2% of total mIgD. Interestingly, significantly more anti-μ (b-7-6) was required to induce desensitization of mIgD. Specifically, ≥1 μg of anti-μ was required for induction of detectable desensitization, an amount of antibody that we estimate saturates >20% of mIgM.

Duration of Anti-Ig-Induced Receptor Desensitization. We next determined the duration of receptor desensitization induced by anti-Ig antibodies. Small B cells were incubated with anti-μ or anti-δ antibodies (10 μg/ml) or no stimulus for 20 min at 37°C before being washed and recultured for from 0.5 to 24 hr. Cells were then harvested and loaded with indo-1, and their Ca^{2+} mobilization response to the appropriate anti-isotypic antibody was assessed. Shown in Fig. 5 are mean $[Ca^{2+}]_i$ values of cells stimulated with anti-μ (b-7-6) 24 hr after stimulation for 20 min with anti-δ (JA12.5) or anti-H-2K (M1.42.3.9.8). The anti-μ response of control cells cultured 24 hr without stimulus is also shown. These results, which typify findings at all time points with both ligand combinations, demonstrate a much reduced responsiveness to anti-μ even 24 hr after an initial 20-min exposure to anti-δ. Thus, ligand-induced mIg desensitization is long-lived, approaching the normal life-span of small B cells (28).

Analysis of the Roles of Second-Messenger Ca^{2+} and Diacylglycerol in mIg Desensitization. In a variety of systems, receptor desensitization is mediated by protein phosphorylation. Available evidence suggests that PKC, activated by diacylglycerol, and perhaps a Ca^{2+}/calmodulin-dependent kinase, activated by Ca^{2+}, may be important in regulation of mIg-mediated signal transduction (9, 10). To explore the roles of these messenger systems in mIg desensitization, we assessed the ability of the Ca^{2+} ionophore ionomycin and exogenous diacylglycerol (diC_8) to induce desensitization. $[Ca^{2+}]_i$ of indo-1-loaded B cells was analyzed by flow microfluorimetry before and after exposure to 0.3 μM ionomycin, which induced increases in $[Ca^{2+}]_i$ comparable to that induced by anti-μ (Fig. 6*A*). After 4–5 min, cells were stimulated with anti-δ and the ensuing Ca^{2+} mobilization response was monitored. Cells exposed to the Ca^{2+} ionophore responded normally to anti-δ (Fig. 6*A*). Thus a Ca^{2+} signal alone appears insufficient to induce mIg desensitization.

Alternatively, $[Ca^{2+}]_i$ of indo-1-loaded cells was analyzed after exposure to diC_8 (2.5 μg/ml) and subsequent stimulation with anti-δ. Diacylglycerol prestimulation reduced the Ca^{2+} mobilization response to anti-δ by ≈80% (Fig. 6*B*). These data suggest that PKC activation leads to desensitization of mIg in B cells, as also suggested by observations that phorbol diesters can induce mIg desensitization (9, 10, 29).

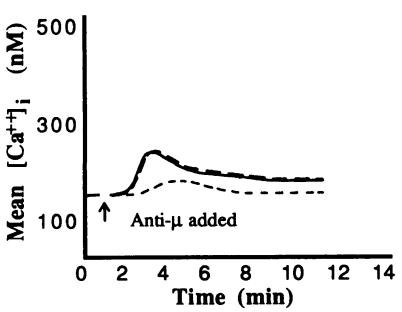

FIG. 5. Ligand-induced desensitization persists for >24 hr. Small B cells (2×10^6) were cultured for 20 min without stimulus (—) or with 10 μg of anti-γ (JA12.5) (---) or anti-$H2^k$ (M1.42.9.8) (—) $F(ab')_2$ before being washed twice and recultured. After 24 hr in culture the cells were harvested and loaded with indo-1, and their mean $[Ca^{2+}]_i$ response to anti-μ (b-7-6, 10 μg/ml) was assessed. Results are representative of three replicate experiments.

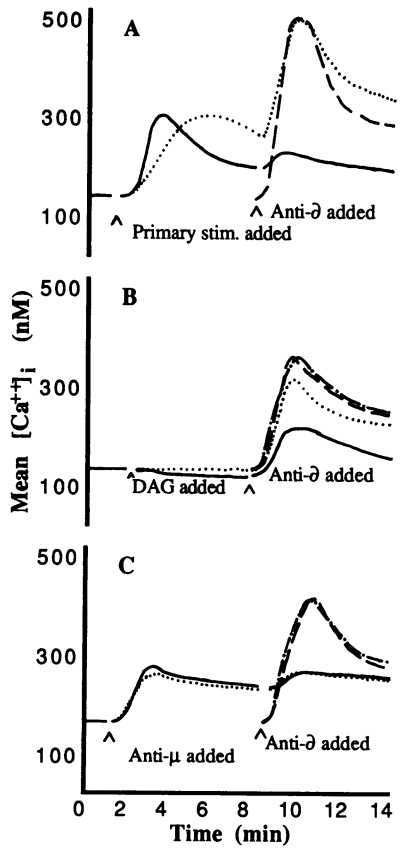

FIG. 6. Second messengers and mIg desensitization. (*A*) Indo-1-loaded small B cells were analyzed before and during culture with no primary stimulus (--), with anti-μ (b-7-6, 10 μg/ml, —), or with 0.3 μM ionomycin (·····) followed by stimulation with anti-δ (JA12.5, 1.0 μg/ml). (*B*) Indo-1-loaded small B cells were incubated without stimulus (--) or with diacylglycerol (DAG; diC_8, 2.5 μg/ml, —) before responsiveness to anti-δ (1.0 μg/ml) was assessed. Alternatively, cells were preincubated 10 min with 20 nM staurosporine before incubation without stimulus (-·-) or with diC_8 (2.5 μg/ml, ·····). (*C*) Indo-1-loaded cells were preincubated for 10 min with (·····, -·-) or without (—, —) 20 nM staurosporine before stimulation with anti-μ followed by anti-δ (—, ·····) or stimulation with only anti-δ (--, -·-). Results are representative of four replicate experiments.

The PKC Inhibitor Staurosporine Inhibits diC_8-Induced, but Not Anti-Ig-Induced, mIg Desensitization. To determine whether PKC mediates anti-Ig-induced desensitization of mIg, we assessed the ability of staurosporine, a potent PKC inhibitor (30), to block the diC_8- and anti-μ-induced desensitization of the anti-δ-stimulated Ca^{2+} mobilization response. B cells loaded with indo-1 were preincubated with or without staurosporine (20 nM) for 10 min before initiation of $[Ca^{2+}]_i$ analysis and exposure to anti-μ and subsequently anti-δ antibodies. Staurosporine blocked diC_8-induced desensitization of the Ca^{2+} mobilization response to anti-δ but did not block anti-μ-induced desensitization. These data indicate that anti-Ig-induced desensitization is mediated at least in part by a PKC-independent mechanism.

DISCUSSION

These studies have directly assessed the ability of mIg molecules to function as signal transducers after the cell's initial interaction with mIg-binding ligands. The data show that ligand binding to as few as 2% of mIg molecules renders the remaining mIg molecules unable to transduce signals leading to Ca^{2+} mobilization and PKC translocation. This

desensitization does not reflect mIg modulation or sequestration of mIg in a site inaccessible to ligand, since anti-μ- or anti-δ-desensitized cells bind the reciprocal antibody normally. Desensitization was detectable >24 hr after an initial interaction with ligand, consistent with the possibility that desensitization lasts for the lifetime of the cell (28). The results further indicate that although diacylglycerol generated after mIg crosslinking is capable of mediating mIg desensitization by activating PKC, it is not the primary mediator of desensitization induced by mIg-crosslinking ligands. Specifically, data presented here demonstrate that exogenous diacylglycerol (diC_8) induces mIg desensitization and that this induction is blocked by the PKC inhibitor staurosporine (30). However, staurosporine does not inhibit anti-μ-induced desensitization of mIgD. In parallel studies, no inhibition of anti-μ induction of mIgD desensitization was seen with other PKC inhibitors including H7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine] and sphingosine (data not shown). Further, diC_8-induced desensitization is of shorter duration than anti-Ig-induced desensitization, being undetectable 24 hr after induction (data not shown). PKC inhibitors also do not appear to effect homologous receptor desensitization reflected by the fall in $[Ca^{2+}]_i$ that occurs within 90–120 sec of stimulation of B cells with anti-μ or anti-δ (Fig. 6 *B* and *C*), suggesting that PKC does not mediate this homologous mIg desensitization.

Ligand-induced receptor desensitization has been reported in a number of systems but is perhaps best documented in the case of the β-adrenergic system (for review see ref. 6). Binding of ligand to β-adrenergic receptors leads to activation of adenylate cyclase and activation of a specific kinase that appears to phosphorylate and thereby desensitize receptors. Recent evidence indicates that under appropriate circumstances, PKC activation also leads to β-adrenergic receptor desensitization. Based on data presented here, on previous findings by Mizuguchi *et al.* (10, 29) that indicate that mIg can be uncoupled from inositolphospholipid hydrolysis by activation of PKC with exogenous phorbol diesters, and on evidence that diacylglycerol is generated as a result of mIg crosslinking (13, 31), it seemed logical to assume that anti-μ- or anti-δ-induced desensitization is mediated in part by PKC. However, data presented here indicate that crosslinking of mIg leads to activation of an alternative mechanism, perhaps a receptor-specific kinase analogous to the β-adrenergic receptor kinase, that is of primary importance in regulation of receptor immunoglobulin function.

Because the molecular basis of the coupling of mIg to inositolphospholipid hydrolysis is not known, it is difficult to formulate a hypothesis to explain mIg desensitization. It is extremely unlikely that receptor sensitivity is regulated by phosphorylation of mIg itself, since the cytoplasmic portions of mIgM and mIgD molecules are only three amino acids long and do not contain the usual phosphate-acceptor residues serine, threonine, or tyrosine. Recent evidence indicates that mIg may transduce signals through a receptor-associated transducer protein analogous to the T3 (CD3) complex expressed by T cells (ref. 32; M. K. Newell, J.C., and M. H. Julius, unpublished data) and a GTP-binding (G) protein (33). Clearly, multiple events intervene between ligand binding to mIg and PKC translocation and Ca^{2+} mobilization. Minimally, these may include mIg association with a transducer complex, transducer association with a G protein, G-protein α-subunit dissociation, α-subunit activation of phospholipase C, and phospholipase C hydrolysis of inositolphospholipids. Any of these steps could be the immediate target of the desensitizing event. Precise characterization of the molecular basis of ligand-induced mIg desensitization must await characterization of these more immediate aspects of the mIg-activated signaling cascade.

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