Cross-Linking of IgG Receptors Inhibits Membrane Immunoglobulin-stimulated Calcium Influx in B Lymphocytes

Daniel Choquet,* Michel Partiseti,* Sebastiàn Amigorena,† Christian Bonnerot,‡ Wolf H. Fridman,‡ and Henri Korn*

* Laboratoire de Neurobiologie Cellulaire, Institut National de la Santé de la Recherche Médicale (INSERM) U261, Institut Pasteur, 75724 Paris, Cedex 15 France; and ‡ Laboratoire d’Immunologie Cellulaire et Clinique, INSERM U255, Institut Curie, 75231 Paris, Cedex 05 France

Abstract. By cross-linking membrane immunoglobulins (mlg), the antigenic stimulation of B lymphocytes induces an increase in intracellular free calcium levels ([Ca2+]i) because of a combination of release from intracellular stores and transmembrane influx. It has been suggested that both events are linked, as in a number of other cases of receptor-induced increase in [Ca2+]i. Conversely, in B lymphocytes, type II receptors for the Fc fragment of IgG (FcγRII) inhibit mlg-mediated signaling. Thus, we have investigated at the level of single cells if these receptors could act on specific phases of mlg Ca2+ signaling.

Lipopolysaccharide-activated murine B splenocytes and B lymphoma cells transfected with intact or truncated FcγRII-cDNA were used to determine the domains of FcγRII implicated in the inhibition of the Ca2+ signal. [Ca2+]i was measured in single fura-2-loaded cells by microfluorometry. The phases of release from intracellular stores and of transmembrane influx were discriminated by using manganese, which quenches fura-2, in the external medium as a tracer for bivalent cation entry. The role of membrane potential was studied by recording [Ca2+]i, in cells voltage-clamped using the perforated patch-clamp method.

Cross-linking of mlgM or mlgG with F(ab’)2 fragments of anti-Ig antibodies induced a sustained rise in [Ca2+]i due to an extremely fast and transitory release of Ca2+ from intracellular stores and a long lasting transmembrane Ca2+ influx. The phase of influx, but not that of release, was inhibited by membrane depolarization. The increase in [Ca2+]i, occurred after a delay inversely related to the dose of ligand. Co-cross-linking mlgS and FcγRII with intact anti-Ig antibodies only triggered transitory release of Ca2+ from intracellular stores but no Ca2+ influx, even when the cell was voltage-clamped at negative membrane potentials. These transitory Ca2+ rises had similar amplitudes and delays to those induced by cross-linking mlgS alone. Thus, our data show that FcγRII does not mediate an overall inhibition of mlg signaling but specifically affects transmembrane Ca2+ influx without affecting the release of Ca2+ from intracellular stores. Furthermore, this inhibition is not mediated by cell depolarization. Thus, FcγRII represents a tool to dissociate physiologically the phases of release and transmembrane influx of Ca2+ triggered through antigen receptors.

Variation in intracellular Ca2+ concentration ([Ca2+]i) is one of the key events in transmembrane signaling. B lymphocytes display on their plasma membrane various receptors which can activate or inhibit Ca2+ signaling, and thus provide a valuable model to study this process. These cells express receptors for antigens which are the membrane-anchored forms of immunoglobulins (mlg). Stimulation by cross-linking mlg, as by a variety of receptors involved in regulation of cell growth, transduces intracellular signals which lead to the activation of phospholipase C and an increase in [Ca2+]i, due to both an initial release from intracellular stores, and a subsequent sustained Ca2+ entry across the plasma membrane (Bijsterbosch et al., 1986; Labaer et al., 1986; Dugas et al., 1987; for review see Rink and Meritt, 1990). The mechanism which underlies Ca2+ release involves, in most cases, activation by inositol 1,4,5-trisphosphate (IP3) of Ca2+ channels present in the membrane of cytoplasmic Ca2+ storage organelles (for review see Berridge and Irvine, 1989). Information about receptor induced Ca2+ influx through the plasma membrane is still very limited, although it probably involves activation of a conductive pathway (Kuno et al., 1986; Lewis and Cahalan, 1989; Hoth and Penner, 1992; Lückhoff and Clapham, 1992). This is mainly due to the lack of defined pharmacological tools and to the difficulty of physiologically dissociating the phase of release from that of influx. It has been suggested in a number of cells, including lymphocytes, platelets, endometrium,
hepatocytes, and exocrine secretory cells, that stimulation of transmembrane influx of Ca2+ is a direct consequence of the emptying of the intracellular IP3-sensitive Ca2+ stores (Scharff et al., 1988; Sage et al., 1989; Takemura, 1989; Putney, 1990; Alvarez et al., 1991; Mason et al., 1991; Meddeleri et al., 1991; Hoth and Penner, 1992). However, the mechanisms underlying the coupling between the state of filling of the store and activation of Ca2+ influx remain unknown and alternative pathways of activation of Ca2+ influx have been proposed. Particularly, IP3 or IP4 receptors linked to Ca2+ permeable channels seem to be present directly in the plasma membrane of lymphocytes (Kuno and Gardner, 1987; Khan et al., 1992b) and endothelial cells (Lückhoff and Clapham, 1992). Thus production of IP3 could directly activate both Ca2+ release and transmembrane Ca2+ influx.

B lymphocytes also express the BL isoform of low affinity receptors for the Fc portion of IgG (FcγRIIb), which inhibit cell activation triggered through mlg (for review see Fridman et al., 1992). This inhibition requires cross-linking of mlg and FcγRII, for example by IgG antibody–covered antigens (immune complexes), and it plays an important role in the negative feedback regulation of antibody production (Phillips and Parker, 1984; Klaus et al., 1987). We and others have shown that co-cross-linking of mlg and FcγRII markedly inhibits the mlg-triggered increase in intracellular Ca2+ (Wilson et al., 1987; Amigorena et al., 1992), and several reports have demonstrated that it decreases IP3 production (Bijsterbosch and Klaus, 1985; Wilson et al., 1987; Rigley et al., 1989). However, inhibition is not complete since the early phases of both Ca2+ and IP3 production seem unaffected by FcγRII (Wilson et al., 1987; Rigley et al., 1989; Amigorena et al., 1992). We thus questioned to what extent the Fc receptors could act specifically on one of the several phases of mlg triggered Ca2+ signaling.

To measure accurately the kinetics of Ca2+ signaling, [Ca2+]i, was recorded by microfluorometry on single, intact or voltage-clamped, fura-2–loaded cells during cross-linking of mlg alone or mlg and FcγRII. Using these methods, we analyzed the capacity of naturally occurring FcγRII-1b and FcγRII-1b-CT76 to express 1–4 × 105 molecules per cell (Amigorena et al., 1992). FcγRIIb-CT77 and FcγRIIb-CT77 are receptors deleted in the cytoplasmic portion of FcγRII. They have been constructed by introducing a stop codon at positions 80 and 77 of the cytoplasmic tail as described previously (Bonnerot et al., 1992). Surface expression of FcγRIIb-CT79 and FcγRIIb-CT76 has been quantified by flowcytometry using the anti-FcγR mAb 2.4G2 (Unkeless, 1979) and was found to be similar to that of the wild-type FcγRIIb. All the experiments presented were performed on clones in which the FcγRII expression was regularly checked using this procedure. Cells were cultured in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated FCS (J BIO), 10 mM glutamine, 20 mM pyruvate, 10 U/ml penicillin, 100 μg/ml streptomycin (Gibco), and 5 × 10−6 M mercaptoethanol (Sigma Chem. Co.).

**Materials and Methods**

**Cells**

Purification of resting B lymphocytes from spleens of 8–10 wk old B6D2Fl mice was performed as previously described (Amigorena et al., 1990). Lipopolysaccharide (LPS)-activated blastocytic cells were obtained by culturing these cells 48–72 h at a concentration of 5 × 105 cells/ml in RPMI 1640 (Gibco, Paisley, England) supplemented with 10% heat-inactivated FCS (J BIO, Paris, France), 1 mM glutamin, 1 mM pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), 2 × 10−3 M mercaptoethanol (Sigma Chem. Co., St. Louis, MO), 25 μg/ml LPS (Difco, Detroit, MI), and incubated at 37°C in 5% CO2 atmosphere.

The IIA1.6 cell line (Jones et al., 1986) is a somatic variant of the A20/2J B lymphoma that has deletion of the 5′ part of the FcγRII gene (Lewis et al., 1986; Bonnerot et al., 1991) and does not express FcγRII molecules. By transfecting cDNA encoding the intact or truncated form of FcγRIIb into this cell line, we obtained clones stably expressing 1–4 × 105 FcγRII molecules per cell (Amigorena et al., 1992). FcγRIIb-CT79 and FcγRIIb-CT76 are receptors deleted in the cytoplasmic portion of FcγRIIb. They have been constructed by introducing a stop codon at positions 80 and 77 of the cytoplasmic tail as described previously (Bonnerot et al., 1992). Surface expression of FcγRIIb-CT79 and FcγRIIb-CT76 has been quantified by flowcytometry using the anti-FcγR mAb 2.4G2 (Unkeless, 1979) and was found to be similar to that of the wild-type FcγRIIb. All the experiments presented were performed on clones in which the FcγRII expression was regularly checked using this procedure. Cells were cultured in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated FCS (J BIO), 10 mM glutamine, 20 mM pyruvate, 10 U/ml penicillin, 100 μg/ml streptomycin (Gibco), and 5 × 10−6 M mercaptoethanol (Sigma Chem. Co.).

**Reagents**

Surface IgM and IgG were cross-linked, respectively, with affinity purified goat F(ab); anti–mouse IgM, μ chain specific (Cappel-Organon Teknika, Fresnes, France), and affinity purified Fab (Fab); anti–mouse IgG, γ chain specific (Cappel-Organon Teknika). FcγRII and surface IgM or IgG were, respectively, co-cross-linked with intact rabbit anti–mouse IgM, μ chain specific (Pierce, Rockford, IL), and intact rabbit anti–mouse IgG (Cappel-Organon Teknika). The monoclonal antibody 2.4G2 was used to cross-link FcγRII alone. All chemicals were from Sigma Chem. Co.

**Recording of Intracellular Ca2+ Levels**

[Ca2+]i was measured at the level of single cells using the Phocal microfluorometric system (Joyce-Loebel, Cambridge, England). 2–10 × 103 cells per ml were incubated with 1 μM fura-2-acetoxy-methyl-ester (Molecular Probes, Junction City, OR) for 15 min at 37°C in the culture medium. The loaded cells were then plated on poly-ornithine–coated (500 μg/ml, 10 min) glass coverslips. After 10–15 min at room temperature, the medium was replaced by Krebs-Ringer solution containing, in mM: NaCl, 140; KCl, 4; MgCl2, 1; CaCl2, 1; Hepes, 10; glucose, 10; pH adjusted to 7.2 with NaOH. The dish was then placed on the stage of an IM-35 Zeiss inverted microscope and fluorescence levels were measured through a 40× objective at 510 nm with a photo multiplier tube (PM) (Thorn EMI, Cambridge, England). The measuring area was limited to a single cell with a diaphragm (Nikon, France) placed in the optic path before the PM. A computer controlled the rotating filter wheel which was equipped with interference filters centered at 340 and 380 nm and was placed in the light pathway of a 75 W xenon lamp that excited single cells alternatively at these two wavelengths.

The absolute intracellular Ca2+ concentrations were calculated on line by the Phocal software using the formula:

\[ [\text{Ca}^{2+}] = K_{\text{app}} \times \frac{(R - R_{\text{rm}})}{(R_{\text{max}} - R)} \]

where R is the ratio between the fluorescence levels measured using the 340 and 380 nm excitation wavelengths (the phocal system allows us to obtain one measure of [Ca2+], every 160 ms). Rm and Rmax are the ratios obtained at 0 and saturating doses of Ca2+, respectively, while Kapp is the apparent dissociation constant for the fura-Ca2+ complex, which depends on the optical set up. Rm, Rmax, and Kapp equaled 0.26, 8.5, and 10 μM, respectively, and were calculated from measurements obtained from cells whole cell patch-clamped with pipette solutions buffered, at known Ca2+ concentrations, with EGTA or containing 10 mM Ca2+. For experiments, anti-Ig antibodies were diluted in Krebs-Ringer solution at the indicated concentrations and applied by pressure ejection through a glass pipette (5–10 μm diam tip, 1.5 mm glass capillary) positioned 10–20 μm from the recorded cell. A continuous suction was applied through a second pipette (50–100 μm diam tip) positioned on the opposite side of the cell to limit diffusion of the antibody containing solution.

**Perforated Patch-Clamp Recordings**

Lymphocytes loaded with fura-2 as above were used. Patch electrodes (5–10 ΩMegaohms) were pulled from soft glass and coated with Crown strip wax (SS white Ltd., Kingston, UK). The bath solution was composed of the following: 140 mM NaCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM Heps, 10 mM glucose, pH 7.2 adjusted with NaOH. The pipette solution contained: 130 mM CsOH, 15 mM CaCl2, 10 mM NaCl, 1 mM CaCl2, 5 mM MgCl2, pH 7.2 adjusted with Methanesulfonate. Just before the experiment, amphotericin B (Sigma Chem. Co.), in stock solution at 30 μg/ml in DMSO, was dissolved in the pipette recording solution at 30 μg/ml. For recordings, the...
tip of the patch electrode was filled with a solution free of amphotericin by capillarity and backfilled with the amphotericin containing solution. After seal formation, the access resistance was monitored through compensation by an Axopatch 1-A (Axon Instrs., Foster City, CA) of the capacitative artifact produced by a 10-mV hyperpolarizing voltage pulse. Typically, an access resistance of 10–30 GΩ was attained after 4–8 min. The input resistance of the cell was always >5 GΩ. These electrical properties ensured that the cell was adequately voltage-clamped. The pipette voltage was imposed through the Axopatch 1-A, and current was monitored on a 2230 digital oscilloscope (Tektroniks, Paris, France). Simultaneous acquisition of voltage, current and fura-2 fluorescence traces was performed by the Phocal Software.

Data Analysis

All data were analyzed on a 386/25 computer (Compaq, Paris, France) using 123 spreadsheet (Lotus Corporation, Paris, France). The delay of the Ca$^{2+}$ response was taken as the time between the onset of the addition of ligand and the point where [Ca$^{2+}$]$_i$ had reached a value of three times that of the baseline level. The half decay time of the Ca$^{2+}$ responses was measured as the time required for the Ca$^{2+}$ level to decrease from its peak value to half of this maximum. All mean experimental values are given ± SD. The relative total cell fura-2 concentration was used to assess quenching of fura-2 by Mn$^{2+}$ and was derived from measurements of fluorescence intensities measured at 340 and 380 nm excitation as follows:

If we assume a one to one binding reaction between fura-2 and Ca$^{2+}$ with an effective dissociation constant:

$$K_d = \frac{\beta}{\alpha}$$

where Furafree + Ca$^{2+}$ $\rightleftharpoons$ Furabound

where Furafree is the free form of fura-2 and Furabound the form bound to Ca$^{2+}$. According to Grynkiewicz et al. (1985), for a mixture of free and Ca$^{2+}$ bound fura-2, at the respective concentrations $[L]$, the total fluorescence intensities at wavelengths 1 and 2 are:

$$F_1 = S_{S1} \cdot [Furafree] + S_{S0} \cdot [Furabound]$$

$$F_2 = S_{S0} \cdot [Furafree] + S_{S2} \cdot [Furabound]$$

Solving Eqs. 1 and 2 for [Furafree] and [Furabound] leads to:

$$[Furafree] = \frac{1}{d} (F_1 \cdot S_{S0} - F_2 \cdot S_{S1})$$

$$[Furabound] = \frac{1}{d} (-F_1 \cdot S_{S2} + F_2 \cdot S_{S0})$$

where $d$ is the discriminant of the system of Eqs. 1 and 2 and equals

$$S_{S0} \cdot S_{S2} - S_{S1} \cdot S_{S0}.$$

Thus, the cell fura-2 concentration $T$ is:

$$T = [Furafree] + [Furabound] = \frac{1}{d} (F_1 \cdot (S_{S0} - S_{S2}) + F_2 \cdot (S_{S1} - S_{S0}))$$

Since $R_{min} = \frac{S_{S0}}{S_{S2}}$, $R_{max} = \frac{S_{S1}}{S_{S0}}$, and $K_{app} = \frac{S_{S0}}{S_{S2}}$ $\cdot$ $K_d$

this relationship can be written:

$$T = \frac{S_{S0}}{d \cdot R_{max}} \cdot (F_1 \cdot (1 - K_{app}) + F_2 \cdot (K_{app} \cdot R_{min} - R_{max}))$$

$R_{min}$ and $R_{max}$ were determined experimentally as described above. $K_{app}$ was determined as the value for which the total cell fluorescence did not vary during a rapid change in [Ca$^{2+}$]$_i$ (in absence of Mn$^{2+}$). This theoretically derived value was always very close to that determined experimentally.

This equation gives $T$ with an unknown multiplicative coefficient $S_{S0}/d$ which was fixed arbitrarily.

Results

FcγRII-Mediated Shortening of mIg-triggered Ca$^{2+}$ Signals

Cross-linking of mIgs by multivalent antigen was mimicked by the use of F(ab')2 fragments of anti-mIgs while co-cross-linking of mIgs and FcγRII by immune complexes was mimicked by intact IgG anti-mIgs antibodies (Phillips and Parker, 1984). Two types of lymphocytes were used which express different mIg isotypes although both express FcγRIIb1i. (1) Splenic B lymphocytes activated during 48 h with LPS which express mIgM (Coutinho and Forni, 1982) and FcγRIIb1i (Amigorena et al., 1989). (2) Clonal cell lines, derived from the FcγRII-defective IIA1.6 B-cell lymphoma (Jones et al., 1986) which express functional membrane IgG2a (Justement et al., 1989; Amigorena et al., 1992), and obtained after stable transfection with the cDNA encoding intact or truncated forms of the FcγRIIb1i (see Materials and Methods).

Fig. 1 shows that cross-linking mIgM on LPS-activated splenic B lymphocytes or mIgG on IIA1.6 cells transfected with FcγRIIb1i induced a sharp rise in [Ca$^{2+}$]$_i$ which remained elevated for hundreds of seconds. Conversely, co-cross-linking FcγRIIb1i and mIgM or mIgG only induced a transitory (15–30 s) rise in [Ca$^{2+}$]$_i$ (Fig. 1). Both the rise time of the Ca$^{2+}$ increase and the delay with which it occurred after addition of the ligand were similar when mIgs alone or mIg and FcRs were cross-linked. The rise occurred with a 20–40 s delay after addition of the ligand to the cell (respectively, 25 s; SD = 16 s; $n = 32$ for 50 μg/ml F(ab')2 anti-IgG and 30 s; SD = 11 s; $n = 39$ for 75 μg/ml intact anti-IgG, measured on FcγRIIb1i transfected cells) and was extremely fast since in most of the cells tested, the peak in [Ca$^{2+}$]$_i$ was reached in < 2–3 s. The rise speed during this explosive period was on the average of 1 μM/s and it was similar in LPS-treated and IIA1.6 cells. The peak value reached by [Ca$^{2+}$]$_i$ was not significantly different under both experimental conditions (respectively, 0.75 μM; SD = 0.39 μM; $n = 32$ for F(ab')2 anti-IgG and 0.70 μM; SD = 0.29 μM; $n = 39$ for intact anti-IgG, measured in FcγRIIb1i transfected cells).

When mIgs alone were cross-linked, after this short phase of sharp rise, [Ca$^{2+}$]$_i$ remained elevated for a length of time which varied from cell to cell and ranged between 100 s and more than 15 min ($mean = 312 s; SD = 147 s; n = 32$, measured on FcγRIIb1i transfected cells). By contrast, when mIgs and FcγRII were cross-linked, [Ca$^{2+}$]$_i$ returned rapidly to baseline levels (mean half-decay time 28.5 s; SD = 210 s; $n = 39$), even slightly lower in some cases, and remained low for the rest of the experiment. This dramatic shortening of the length of the Ca$^{2+}$ response by FcγRII engagement in both cell types is quantified in Fig. 2.

To confirm the involvement of the Fc receptors in this inhibitory process, we performed similar experiments after preincubation of the cells with a rat monoclonal antibody, 24G.2 (Unkeless, 1979), against FcγRII. As shown in the bottom set of traces in Fig. 1, using these conditions, the
do not hallucinate.

 RAW TEXT START
 Page dimensions: 612.0x792.0

[Image 0x0 to 612x792]

Tact anti-mIg. They also confirm that separate occupancy of mIgM or mIgG-mediated Ca²⁺ responses triggered by in-

under control conditions (,,) and after 2-10 rain preincubation

cross-linking mIg with or without FcyRII. Rises in [Ca²⁺]i were

suggest that FcyRIIs are responsible for the inhibition of the

with a mouse anti-rat antibody (not shown). These data

Figure (Fig. 2). Addition of 24G.2 alone did not induce a rise in

induced by F(ab')² fragments (ta) or intact anti-immunoglobulins

[Ca²⁺]. even after the Fc-yRII cross-linking was augmented

in Fig. 1 and are expressed as mean ± SD of 8-39 cells.

Figure 3 shows that b1CT79, a mutant receptor with 16 amino

acyl acid cytoplasmic tail, of 47 amino acids at a site proximal
to the membrane. We had previously found that the b2 iso-

form differs from FcyRIIb1 by deletion, in the 94-amino

acid cytoplasmic tail. This is not necessary for inhibition. We thus questioned which of

the 47 amino acids common to the cytoplasmic tail of bl and
b2 are necessary to inhibit mlg signaling by analyzing the
effect of deletions in the cytoplasmic tail of the b1 isoform.

Fig. 3 shows that b1CT79, a mutant receptor with 16 amino

acids deleted from the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

FcγRII and mlgG does not prevent signaling by mlgs, and
that co-cross-linking of both receptors is necessary for inhibi-
tion (Klaus et al., 1987).

We further analyzed the mode of operation of FcyRIIb1 by
studying which structural parts of the receptor are involved in inhibition of mlg signaling. Because of alternative mRNA
splicing, a second isoform of FcyRII, termed FcyRIIb2, is
expressed in myeloid cells, but normally not in B lympho-
cytes (Lewis et al., 1986; Amigorena et al., 1989). This iso-

form differs from FcyRIIb1 by deletion, in the 47-amino

acid cytoplasmic tail, of 47 amino acids at a site proximal
to the membrane. We had previously found that the b2 iso-

form of FcyRII is also able to inhibit mlg-mediated activa-
tion, while a mutant receptor in which the intracellular tail is

shortened to the 47-amino acid insert specific to the FcyRIIb1 sequence is not functional (Amigorena et al.,
1992). These data established that this 47-amino acid insert
is not necessary for inhibition. We thus questioned which of

the 47 amino acids common to the cytoplasmic tail of bl and
b2 are necessary to inhibit mlg signaling by analyzing the
effect of deletions in the cytoplasmic tail of the b1 isoform.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.

Dose-Dependence of F(ab')2 and Intact anti-mlgG

FIGURE 3. Disruption of FcγIRI-

Rb1 function by deletion of

19-amino acids deletion in the COOH terminal of the cyto-

plasmic tail. Only b1CT79 is capable of inhibiting Ca²⁺ signaling
mediated by mlgs.
dose-dependent decrease in the mean peak [Ca\textsuperscript{2+}] amplitude and in the percentage of cells which gave a response. However, at all doses [Ca\textsuperscript{2+}] remained elevated for several minutes after the initial response.

Similar results were obtained when the dose of intact anti-IgG was changed (not shown). As the dose of anti-IgG was lowered, we observed an increase in the delay between the addition of the ligand and the start of the rise in [Ca\textsuperscript{2+}]. (top), the mean peak amplitude of [Ca\textsuperscript{2+}], value (middle) and the percentage of cells in which addition of the ligand induced a rise in [Ca\textsuperscript{2+}]. (bottom). Data are expressed as mean ± SD of 7-32 cells. The protocol used for measurements is described in Materials and Methods.

**Involvement of Both Ca\textsuperscript{2+} Release from Intracellular Stores and of Influx through the Plasma Membrane in the Generation of F(ab')\textsuperscript{2}-triggered [Ca\textsuperscript{2+}]-Increases**

We analyzed the origin (extra-versus intracellular) of Ca\textsuperscript{2+} during the mIg-mediated increase in [Ca\textsuperscript{2+}], by removing extracellular Ca\textsuperscript{2+} or by depolarizing the cell with KCl at different times with respect to the addition of F(ab')\textsuperscript{2} anti-IgG. When these manipulations were performed later than one minute after the onset of stimulation, we observed a systematic decrease of [Ca\textsuperscript{2+}], back to baseline levels (Fig. 5, A1 and B1). In contrast, when the cells were stimulated in the absence of extracellular Ca\textsuperscript{2+} or in the absence of KCl (Fig. 5, A2 and B2), there were still sharp rises in [Ca\textsuperscript{2+}], with a delay similar to those occurring in control experiments. However, [Ca\textsuperscript{2+}] rapidly returned to baseline levels after this initial response. The decrease in [Ca\textsuperscript{2+}] induced by either removing extracellular Ca\textsuperscript{2+} or by adding KCl was reversed upon switching back to a Ca\textsuperscript{2+} and NaCl containing extracellular solution in most experiments (respectively, 26 out of 31 and 20 out of 34) thus showing that the Ca\textsuperscript{2+} influx pathway was still functional (Fig. 5, A2 and B2). Addition of the K\textsuperscript{+} ionophore valinomycin (1 μM) to the solutions in order to clamp the transmembrane potential at the K\textsuperscript{+} equilibrium potential did not modify the percentage of cells which recovered from the KCl mediated inhibition (24 out of 40 cells recovered in these conditions). This shows that the lack of reincrease of [Ca\textsuperscript{2+}], in certain cells upon switching back to the NaCl containing medium after depolarization with KCl is not due to an absence of repolarization, but rather to a closure of the influx pathway.

Altogether our data show that cross-linking mIgGs induces a rise in [Ca\textsuperscript{2+}], due to an early voltage-independent release of Ca\textsuperscript{2+} from intracellular stores and an influx of Ca\textsuperscript{2+} through the plasma membrane which is inhibited by membrane depolarization.

**Selective Inhibition of mIg-triggered Ca\textsuperscript{2+} Influx by Fc\gammaRII Engagement**

Co-cross-linking mIg and Fc\gammaRII triggered only a short and sharp increase in [Ca\textsuperscript{2+}]. This already suggested that this response is mainly the consequence of release of Ca\textsuperscript{2+} from
Intracellular stores. However other mechanisms, such as activation by FcγRII of a Ca<sup>2+</sup> extrusion pump could also have accounted for the rapid return of [Ca<sup>2+</sup>], to baseline levels. To discriminate between these different hypotheses, we directly assessed the opening of a transmembrane Ca<sup>2+</sup> influx pathway by using manganese (Mn<sup>2+</sup>) as a tracer for divalent cation entry. Mn<sup>2+</sup> binds to fura-2 and quenches its fluorescence (Gryniewicz et al., 1985). Thus, when the extracellular medium contains Mn<sup>2+</sup>, activation of a Mn<sup>2+</sup>-permeable Ca<sup>2+</sup> influx pathway leads to Mn<sup>2+</sup> influx, and consequently to a decrease in total cellular fura-2 fluorescence (Hallam et al., 1989; Sage et al., 1989; Alvarez et al., 1991; Lückhoff and Clapham, 1992). It must be noted that not all the receptor-activated Ca<sup>2+</sup> influx pathways allow Mn<sup>2+</sup> as a permeant cation (Merritt and Hallam, 1988; Hoth and Penner, 1992). Total cellular fura-2 fluorescence must be measured using conditions where it does not depend on [Ca<sup>2+</sup>]. The commonly used experimental approach consists of measuring cell fluorescence at the isosbestic wavelength for fura-2 excitation (i.e., the wavelength at which fura-2 fluorescence is independent of Ca<sup>2+</sup> concentration, around 360 nm). Simultaneous recordings of [Ca<sup>2+</sup>], and cell fluorescence have been previously performed using either three wavelength (two for [Ca<sup>2+</sup>], one at the isosbestic point), or only two, one being the isosbestic wavelength. In the latter case, there is a loss in the dynamic range over which varies the fluorescence ratio used to calculate [Ca<sup>2+</sup>]. These problems were avoided by calculating the theoretical fura-2 concentration from fluorescence intensity measurements at 340 nm and 380 nm excitation wavelengths (see Materials and Methods).

Experiments were performed by stimulating the cells in a medium containing no Ca<sup>2+</sup> and 0.5 mM Mn<sup>2+</sup>. Control experiments showed that these conditions do not lead to spontaneous decrease in cell fluorescence, indicating that these cells have a low baseline membrane permeability to Mn<sup>2+</sup>. Fig. 6A shows simultaneous measurement of [Ca<sup>2+</sup>], and total cell fluorescence in two FcγRIIB-Iβ cells challenged with F(ab)2 and intact anti-IgG antibodies. After a short delay both stimuli induced a transitory sharp rise in [Ca<sup>2+</sup>], due solely to release of Ca<sup>2+</sup> from intracellular stores since there was no extracellular Ca<sup>2+</sup>. However, while cross-linking IgG alone led to a rapid quenching of cell fluorescence (n = 10 cells), virtually no quenching was observed when IgG and FcγRIIB were co-cross-linked (n = 11 cells). The mean percentages of fluorescence quenching in both experimental conditions are given in Fig. 6B. As in the experiments illustrated by Fig. 5, A1 and A2, readdition of Ca<sup>2+</sup> to the extracellular medium several minutes after the onset of stimulation with F(ab)2 induced a new increase in [Ca<sup>2+</sup>], thus confirming that the Ca<sup>2+</sup> influx pathway was still open. This protocol temporarily stopped fluorescence quenching since Mn<sup>2+</sup> was absent during this period. In contrast, reintroduction of extracellular Ca<sup>2+</sup> never led to a rise in [Ca<sup>2+</sup>], in cells stimulated with intact anti-IgG. These data indicate that the Ca<sup>2+</sup> influx pathway activated through IgG is permeable to Mn<sup>2+</sup> and inhibited by engagement of FcγRIIB.

Interestingly, in all cells tested with F(ab)2, quenching started within a few seconds after the sharp release of Ca<sup>2+</sup> from intracellular stores and developed quasi-linearly during the rest of the experiment. This indicates that the transmembrane influx of Ca<sup>2+</sup> is activated shortly after the cytoplasmic release. Moreover, the amount of Ca<sup>2+</sup> released from intracellular stores was only slightly smaller in intact versus F(ab)2 anti-IgG stimulated cells, as indicated by measurements of the peak amplitudes and half decay times of the Ca<sup>2+</sup> responses triggered in the absence of extracellular Ca<sup>2+</sup> (respectively, 0.56 μM; SD = 0.41 μM and 46 s; SD = 40 s, n = 10 for F(ab)2, and 0.50 μM; SD = 0.34 μM and 38 s; SD = 25 s; n = 9 for intact anti-IgG). This shows that release of intracellular Ca<sup>2+</sup> triggered through IgG is little affected by FcγRIIB.

**FcγRII Inhibition of Ca<sup>2+</sup> Influx Is Not Mediated by Cell Depolarization**

Activation of Ca<sup>2+</sup> influx after cross-linking of IgG requires both opening of a Ca<sup>2+</sup> influx pathway in the plasma membrane and a negative membrane potential to provide the electrical driving force for Ca<sup>2+</sup> entry. Indeed, as shown in Fig. 5, membrane depolarization blocks Ca<sup>2+</sup> influx. We thus questioned whether inhibition of Ca<sup>2+</sup> influx by FcγRII was mediated by cell depolarization. To test this hypothesis, we performed experiments using the perforated patch-clamp method (Horn and Marty, 1988; Rae et al., 1991) which allows us to clamp the transmembrane voltage of cells with minimal perturbation of the cell interior. Fig. 7 shows recordings of [Ca<sup>2+</sup>], in two FcγRIIB-Iβ cells voltage-clamped at negative and positive membrane potentials. In control conditions, [Ca<sup>2+</sup>], remained low in cells voltage-clamped at −70 mV, which is close to the normal resting potential of lymphocytes (Deutsch et al., 1979; Rink et al., 1980). Cross-linking
mlg induced a sustained rise in \([\text{Ca}^{2+}]_i\), which was reversibly inhibited by membrane depolarization \((n = 5 \text{ cells})\). At the opposite, co-cross-linking mlg and FcγRII only produced a transient rise in \([\text{Ca}^{2+}]_i\), even though the cell was voltage-clamped at \(-70 \text{ mV}\). After this initial rise in \([\text{Ca}^{2+}]_i\), which mostly originated from release of Ca 2+ from intracellular stores \(\text{see above}\), \([\text{Ca}^{2+}]_i\), remained low for the rest of the experiment. Similar results were obtained in seven cells. Thus, maintaining a negative membrane potential does not prevent inhibition of Ca 2+ influx by FcγRII. This indicates that the mode of action of this receptor does not involve membrane depolarization.

**Discussion**

By measuring \([\text{Ca}^{2+}]_i\), in single cells, we have shown that \((a)\) cross-linking mlg triggers an increase in \([\text{Ca}^{2+}]_i\), due to an explosive and transitory release of Ca 2+ from intracellular stores and a sustained influx through the membrane, and \((b)\) co-cross-linking mlg and FcγRII specifically inhibits the influx component, without affecting the release phase.

**mlg Triggered Ca 2+ Responses**

Although a number of studies have analyzed the changes in \([\text{Ca}^{2+}]_i\), which occur upon mlg cross-linking \(\text{Braun et al., 1979; Pozzan et al., 1982; Bijterbosch et al., 1986; Labaer et al., 1986; Ransom et al., 1986; Dugas et al., 1987; MacDougall et al., 1988; Ransom et al., 1988; for review see DeFranco et al., 1987; Cambier et al., 1987},\) no details on their kinetics were available up to now since all, but one \(\text{Wilson et al., 1987}\) of the published investigations involved populations of a large number of cells. New information provided in this study on single cells indicates that Ca 2+ rises to micromolar levels extremely fast \(\text{sometimes in } < 1 \text{ s}\) with a dose dependent delay of \(10-200 \text{ s}\) after addition of the ligand. This suggests that among the numerous steps which lie between mlg cross-linking and Ca 2+ release, some initial events are rate limiting while the increase in \([\text{Ca}^{2+}]_i\), is controlled by a strongly regenerative process.

The biochemical mechanisms which underlie Ca 2+ release have been extensively studied and several examples of strong positive cooperativity have been reported. For example, both IP 3 mediated Ca 2+ release and phospholipase C activity, which generate IP 3, are potentiated by elevated \([\text{Ca}^{2+}]_i\), \(\text{Finch et al., 1991; Harootunian et al., 1991; Smrcka et al., 1991}\) and could thus participate in a regenerative mechanism. Since the delay of the responses reaches finite and nonzero asymptotic values as the doses of ligand are respectively lowered or increased \(\text{see Fig. 4 }B\), there must be some constraints upon the mechanisms by which Ca 2+ responses are generated. One possible model is that mlg triggers the formation of a second messenger X, likely to be IP 3 \(\text{Coggeshall andCambier, 1984; Bijterbosch et al., 1985; Ransom et al., 1986; Fahey and DeFranco, 1987; Ransom et al., 1988}\) in a ligand-dose dependent manner \(\text{McConnell et al., 1992}\). The nonzero lower limit of the delay suggests that the intracellular concentration of X must reach a threshold value before the regenerative mechanism of Ca 2+ release is triggered. The higher limit of the delay may be accounted for if X is permanently degraded at a fixed rate, independent of cell activation \(\text{by an IP 3-phosphatase, for example}\). According to these hypotheses, the doses of ligand which trigger formation of X at a lower rate than its degradation, would never produce a response, while the amplitude of the latter should be dose independent. Such a mechanism has been proposed to account for a similar behavior of PAF induced Ca 2+ responses in macrophages \(\text{Randriamampita and Trautmann, 1989}\). However, this model does not fully account for our data, since we observed a decrease in the amplitude of the Ca 2+ response at very low doses of ligand, thus showing that the process is not entirely all or none.

**FcγRIIb Mediated Inhibition of Ca 2+ Influx**

Our data show that intact antibodies to mlgG or mlgM induce increases of intracellular Ca 2+ much shorter in duration than those triggered by F(ab')2 fragments of anti-IgG or -IgM. Two main arguments indicate that this is due to an inhibition by FcγRII of Ca 2+ signaling through mlgG. First, preincubation of the cells with an anti-FcvRII antibody prevented the block of signaling by intact anti-mlgs. Second, in cells transfected with a truncated and inactive form of FcγRIIb, both intact and F(ab')2 anti-mlgs induced increases in \([\text{Ca}^{2+}]_i\), of equally long duration. Furthermore, inhibition occurs at the level of a single lymphocyte and thus does not require cooperation of receptors present on several cells. This conclusion could not be derived from studies performed on entire populations of cells \(\text{Bijterbosch and Klaus, 1985; Wilson et al., 1987; Rigley et al., 1989}\).

The main outcome from our results is that FcγRII does not mediate an overall inhibition of mlg mediated signaling, but specifically affects certain of its aspects. First, by manipulating extracellular Ca 2+ and assessing direct activation of a Ca 2+/Mn 2+ influx pathway, we found that co-cross-linking FcγRII and mlgG totally blocks mlgG-triggered transmembrane...
but only drives them to an intermediate step between Go protein itself. This hypothesis is strengthened by the observation that co-cross-linking of mlgM and FcγRII cross-linked mlgM and FcγRII by immune complexes (Ag-Ab complex) inhibits transmembrane Ca²⁺ influx either by acting on a specific pathway of activation or by shutting off mlg signaling after the initial signals which lead to Ca²⁺ release are delivered.

Ca²⁺ influx, with little or no inhibition of mlg-induced release of Ca²⁺ from intracellular stores. This may account for the fact that cross-linking mlg and FcγRII does stimulate resting (Go) B cells to increase Ia antigen expression, but only drives them to an intermediate step between Go and G₁, while cross-linking mlgM alone induces progression to late resting (Go) B cells to increase Ia antigen expression, while proceeding between the S phase of the cell cycle (Klaus et al., 1984). Second, whatever the doses of ligand used, the rises in [Ca²⁺]i induced by F(ab′)2 fragments of anti-mlgs were always long lasting, while those triggered by intact anti-mlgs were of short duration. This shows that the effect of intact anti-mlgs cannot be mimicked by lowering the dose of F(ab′)2 anti-mlgs. Thus, cross-linking FcγRII and mlg does not solely interfere with an adequate cross-linking of mlg and its subsequent ability to deliver activation signals to intracellular effectors. This conclusion is further supported by the observation that truncation of only three amino acids in the intracellular tail of FcγRIIb disrupts its function.

Thus FcγRII probably acts at a step downstream of the mlg protein itself. This hypothesis is strengthened by the observation that cross-linking of mlgM and FcγRII cross-inhibits signaling through mlgDs present on the same cell (Klaus et al., 1985). Our voltage-clamp data show that the inhibition of Ca²⁺ influx by FcγRII is not due to a decreased transmembrane electrical driving force for this ion, but rather to the lack of opening of the influx pathway itself. Rigley et al. (1989) have shown in permeabilized cells that FcγRII inhibits the GTPyS dependent mlg signaling but not the baseline GTPyS signaling. Thus, they have suggested that FcγRII acts by uncoupling the mlg from an associated G protein. Since that FcγRII does not affect early IP₃ production (Bijsterbosch and Klaus, 1985; Wilson et al., 1987; Rigley et al., 1989) or Ca²⁺ release (this study), several biochemical pathways may be involved in mlg signaling. Along this line, recent studies have characterized tyrosine kinases associated with the mlg complex which can directly activate phospholipase C by tyrosine phosphorylation (Burkhardt et al., 1991; Carter et al., 1991; Yamanashi et al., 1991). Thus it is possible that two independent pathways lead respectively to Ca²⁺ release and transmembrane influx, only the latter being susceptible to inhibition by FcγRII. However, a current hypothesis regarding the mechanism of receptor-induced Ca²⁺ influx is that it is triggered, through an unknown process, by the emptying of the intracellular Ca²⁺ pools (Sage et al., 1989; Takemura, 1989; Putney, 1990; Meldolesi et al., 1991; Hoth and Penner, 1992). In lymphocytes, a transmembrane influx of Ca²⁺ is induced after depletion of these pools, either by loading the cells with a Ca²⁺ chelator or by the Ca-ATPase inhibitor thapsigargin, which empties Ca²⁺ stores by preventing their refilling (Scharff et al., 1988; Alvarez et al., 1991; Mason et al., 1991). Reconciliation of our data with the store-dependent-Ca²⁺-influx hypothesis would require that FcγRII inhibit Ca²⁺ influx at a stage downstream from that of release, maybe even directly at the level of the putative Ca²⁺ channel itself. Alternatively, sustained receptor-triggered efflux of Ca²⁺ from the internal pools may be necessary for transmembrane influx to occur (Putney, 1990). This might suggest that FcγRII are initially in an inactive form and allow mlg to trigger production of an early Ca²⁺ releasing signal such as IP₃. These initial steps could then render FcγRII capable of inhibiting signaling through mlg of IP₃ production and thus prevent further release and activation of the influx pathway. It should be noted that this hypothesis holds whether influx is activated by emptying of the stores (Scharff et al., 1988; Sage et al., 1989; Takemura, 1989; Putney, 1990; Alvarez et al., 1991; Mason et al., 1991; Meldolesi et al., 1991; Hoth and Penner, 1992) or through IP₃ receptors in the plasma membrane (Kuno and Gardner, 1987; Khan et al., 1992a,b; Lückhoff and Clapham, 1992).

In summary (Fig. 8), FcγRII provides a tool which allows the physiological dissociation of the phases of Ca²⁺ release and transmembrane influx. This may help to understand how these two processes are linked and will probably involve an as yet unknown mechanism of general cellular interest.

We thank P. Legendre and M. Picciotto for their advice on the manuscript and V. Devignot for excellent technical assistance. This work was supported by grants from the Institut National pour la Santé et la Recherche Médicale, the Association Française contre les Myopathies, and the Délégation Régionale de l’Équipement du Territoire (contract 602.003).

Received for publication 2 October 1992 and in revised form 30 November 1992.

References

Alvarez, J., M. Montero, and J. Garcia-Sacho. 1991. Cytochrome P-450 may link intracellular Ca²⁺ stores with plasma membrane Ca²⁺ influx. Biochem. J. 274:193-197.

Amigorena, S., C. Bonnerot, D. Choquet, W. H. Fridman, and J. L. Teillaud. 1989. FcγRII expression in resting and activated B lymphocytes. Eur. J. Immunol. 19:1379-1385.

Amigorena, S., D. Choquet, J. L. Teillaud, H. Korn, and W. H. Fridman. 1990. Ion channels blockers inhibit B cell activation at a precise stage of the G1 phase of the cell cycle. J. Immunol. 144:2038-2045.

Berridge, M. J., and R. F. Irvine. 1989. Inositol phosphates and cell signaling. Nature (Lond.). 341:197-205.

Bijsterbosch, M. K., and G. G. B. Klaus. 1985. Cross-linking of surface immunoglobulin and Fc receptors on B lymphocytes inhibits stimulation of inositol phospholipid breakdown via the antigen receptors. J. Exp. Med. 162:1825-1836.

Bijsterbosch, M. K., C. J. Meade, G. A. Turner, and G. G. B. Klaus. 1985. B lymphocyte receptors and polyphosphoinositide degradation. Cell. 41:999-1006.

Bijsterbosch, M. K., K. P. Rigley, and G. G. B. Klaus...1986. Cross-linking of
of surface immunoglobulin on B lymphocytes induces both intracellular Ca2+ release and Ca2+ influx: analysis with indo-1.

326:301-304.

Kuno, M., J. Goronzany, M. W. Cornelis, and P. Gardner. 1986. Single-channel and whole-cell recordings of whole-cell-regulated inward currents in human clonally helped T lymphocytes. Nature (Lond.). 323:269-273.

Labrador, J. R., Y. Tsien, K. A. Fahey, and A. L. DeFranco. 1986. Stimulation of the antigen receptor on WEHI-231 B lymphoma cells results in a voltage-independent increase in cytoplasmic Ca2+, J. Immunol. 137:1836-1844.

Lewis, R. S., and M. D. Cahalan. 1989. Mitogen-induced oscillations of cytosolic Ca2+ current in human leukemic T cells. Cell Regulation. 1:99-112.

Lewis, V. A., T. Koch, H. Pluznik, and I. Melfain. 1986. A complementary DNA clone for a macrophage Fc receptor, Nature (Lond.). 324:372-375.

Luczak, A., and D. E. Clapham. 1992. Inositol 1,3,4,5-tetrakisphosphate activates an endothelial Ca2+-permeable channel. Nature (Lond.). 355:356-358.

Masaguall, S. L., S. Grinstein, and E. W. Gelfand. 1988. Detection of ligand-activated conductive Ca2+ channels in human B lymphocytes. Cell. 54:229-234.

Mason, J. M., M. P. Mahaut-Smith, and S. Grinstein. 1991. The role of intracellular Ca2+ in the regulation of the plasma membrane Ca2+ permeability of unstimulated rat lymphocytes. J. Biol. Chem. 266:10872-10879.

McConnell, F. M., S. B. Shears, J. L. Lane, M. S. Scheibel, and E. A. Clark. 1992. Relationships between the degree of cross-linking of surface immunoglobulin and the associated inositol 1,4,5-trisphosphate and Ca2+ signals in human B cells. J. Biol. Chem. 268:445-477.

Meldolesi, J., E. Clementi, C. Fasolato, D. Zacchetti, and T. Pozzan. 1991. Ca2+ influx following receptor activation. Trends in Pharmacol. Sci. 1:289-297.

Merritt, J. E., and T. J. Hallam. 1988. Platelets and parotid acinar cells have different mechanisms for agonist stimulated dvalinlc cation entry. J. Biol. Chem. 263:6161-6164.

Phulan, N. E., and D. C. Parker. 1984. Cross-linking of B lymphocyte Fc receptors and membrane immunoglobulin inhibits anti-immunoglobulin-induced blastogenesis. J. Immunol. 132:627-632.

Pozzan, T., P. Arslan, R. Y. Tsien, and T. J. Rink. 1982. Anti-immunoglobulin-stimulated free Ca2+ and capping in B lymphocytes. J. Cell Biol. 94:335-340.

Putney, J. W. 1990. Capacitative Ca2+ entry revisited. Cell Calcium. 11:1-62.

Rae, J. K., Cooper, G. Gates, and M. Watsky. 1991. Low access resistance perforated patch recordings using amphotericin B. J. Neurosci. Methods. 37:15-26.

Randrianampita, C., and A. Trautmann. 1989. Biphasic increase in intracellular Ca2+ induced by platelet-activating factor in macrophages. FEBs (Fed. Eur. Biochem. Soc.) Lett. 249:199-206.

Ransom, J. T., L. K. Harris, and J. C. Cambier. 1986. Anti-Ig induces release of inositol 1,4,5-trisphosphate which mediates mobilization of intracellular Ca2+ stores in B lymphocyte. J. Immunol. 137:708-713.

Ransom, J. T., M. Chen, V. M. Sandoval, J. A. Pasternak, D. Diiguisto, and J. C. Cambier. 1988. Increased plasma membrane permeability to Ca2+ in anti-Ig-stimulated B lymphocytes is dependent on activation of phosphoinositide hydrolysis. J. Immunol. 140:3150-3155.

Rigley, K. P., M. M. Harnett, and G. B. Klaus. 1989. Co-cross-linking of surface immunoglobulin Fc receptors on B lymphocytes uncouples the anti-Ig receptors from their associated G proteins. Eur. J. Immunol. 19:481-485.

Rink, T. J., and J. E. Merritt. 1990. Ca2+ signaling. Curr. Opin. Cell Biol. 2:198-205.

Rink, T. J., C. Montecucco, T. R. Heikath, and R. Y. Tsien. 1980. Lymphocyte membrane potential assessed with fluorescent probes. Biochem. Biophys. Acta. 595:15-30.

Sage, S. O., J. E. Merritt, J. T. Hallam, and T. Rink. 1989. Receptor-mediated Ca2+ entry in furin-2-loaded human platelets stimulated with ADP and thrombin. Biochem. J. 258:923-926.

Scharff, O., B. Foder, O. Thastrup, B. Hofmann, J. Moiler, L. P. Ryder, K. D. Jacobsen, E. Langhoff, E. Dickmeiss, S. B. Christensen, S. Pinkoj, and A. Sveigaard. 1988. Effect of thapsigargin on cytoplasmic Ca2+ and proliferation of human lymphocytes in relation to AIDS. Biochem. Biophys. Acta. 972:257-264.

Smrcka, A. V., J. R. Hepler, K. O. Brown, and P. C. Sternweis. 1991. Regulation of polyphosphoinositide-specific phospholipase C activity by purified Ca2+ waves. Science (Wash. DC). 254:804-807.

Takemura, H., A. R. Hughes, O. Thastrup, and J. W. Putney. 1989. Activation of Ca2+ entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular Ca2+ pool and not an inositol phosphate regulated Ca2+ fluxes at the plasma membrane. J. Biol. Chem. 264:12260-12271.

Unkeless, J. C. 1979. Characterization of monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med. 150:580-590.

Wilson, H. A., D. Greenblat, M. Poinie, D. F. Finkelman, and R. Y. Tsien. 1987. Crosslinkage of B lymphocyte surface immunoglobulin by anti-Ig or antigen induces prolonged oscillation of intracellular ionized Ca2+. J. Exp. Med. 166:601-606.

Yamanashi, Y., T. Kariuchi, T. Yamasaki, and T. Yoshimura. 1991. Association of B cell antigen receptor with protein tyrosine kinase lyn. Science (Wash. DC). 251:192-194.