Signal Transduction by Neutrophil Immunoglobulin G Fc Receptors

DISSOCIATION OF INTRACYTOPLASMIC CALCIUM CONCENTRATION RISE FROM INOSITOL 1,4,5-TRISPHOSPHATE*

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The signal transduction mechanisms involved in the regulation of phagocytosis are largely unknown. We have recently shown that in neutrophils, when IgG-mediated phagocytosis is stimulated by formyl-methionyl-leucyl-phenylalanine (fMLP), and insoluble immune complexes caused an increase in [Ca2+]. The rise in [Ca2+], induced by Fc receptor ligation was resistant to pertussis toxin. In contrast, fMLP induced a rise in 

\[ \text{[Ca}^{2+}\text{]} \]

which was inhibited by pertussis toxin. fMLP-induced [Ca2+], was accompanied by an accumulation of inositol 1,4,5-trisphosphate (IP3) which peaked by 15 s, and which was also abolished by pertussis toxin. IP3 accumulation after aggregated IgG, 3G8, or insoluble immune complexes was much less than after fMLP. Unlike [Ca2+], rise induced by Fc receptor ligation, this small increase in IP3 was inhibited by pertussis toxin. These data demonstrated that the [Ca2+], increase induced by Fc receptor ligation is not mediated by IP3. Immediate pretreatment of human polymorphonuclear neutrophils with optimal doses of fMLP also reduced subsequent increase in [Ca2+]; rise from thapsigargin, a sesquiterpene lactone tumor promoter that releases intracellular Ca2+ from IP3-sensitive stores without IP3 turnover. Similarly, to its effects on thapsigargin, fMLP inhibited the [Ca2+], rise upon subsequent immune complex binding. Pretreatment of cells with immune complexes also prevented subsequent [Ca2+], rise from thapsigargin and fMLP. These data demonstrate that IgG Fc receptor ligation and fMLP activation of human polymorphonuclear neutrophils use distinct signal transduction mechanisms to release Ca2+ from the same thapsigargin-sensitive intracellular pool. In contrast to fMLP, signal transduction for increased [Ca2+], after Fc receptor stimulation does not involve a pertussis toxin-sensitive G protein, and is independent of IP3.

Antibodies have two major functions: the binding to antigen via their antigen-combining sites and the activation of defense mechanisms via their carboxyl termini, the Fc region. Ligation of the Fc portion to specific receptors on many cells of the immune system triggers several functions including phagocytosis, antibody dependent cell-mediated cytotoxicity, secretion of inflammatory mediators, generation of the respiratory burst, and clearance of immune complexes (1, 2). Because the Fc receptors (FcR) mediate these important defense functions of the immune system, understanding signal transduction from receptor-ligand interaction is a central question in phagocyte biology.

Human phagocytic cells bear at least three distinct types of FcR, all members of the Ig gene superfamily. Knowledge of their structures and gene organization has progressed rapidly in the past few years (3, 4). However, the signals transduced by each of the FcR to activate particular cell responses are not completely elucidated.

Human neutrophils (PMN) express two FcR: FcRII (CD32) which has a traditional membrane spanning domain and a cytoplasmic tail (6, 7) and FcRII (CD16) which has a glycosylphosphatidylinositol anchor (7). FcRII exists as a transmembrane anchored form in NK cells (8). Each of these receptors has been shown to express structural polymorphisms that might be functionally significant (9, 10). FcRII, with a higher density on the cell membrane than FcRII, has been thought to be primarily a binding molecule, used to present ligand to FcRII, which in turn will mediate signaling across the membrane (11, 12). This idea is attractive because the glycosylphosphatidylinositol linkage of FcRII has no obvious signaling mechanism. However, recent reports (13, 14) and our own results (15) indicate that FcRII is able to transduce the signal for several cell responses, including an increase in cytoplasmic free Ca2+ concentration (\([\text{Ca}^{2+}]\)).

Previously, we found that FcR-mediated phagocytosis stimulated by fMLP, but not by phorbol esters or platelet-activating factor, is dependent on an increase in [Ca2+]. (15). Moreover, the [Ca2+] rise which is important for phagocytosis comes from ligation of FcR. Apparently increases in [Ca2+] can occur after ligation of either FcRI or FcRII (14, 15). Signal transduction events leading to increased [Ca2+] have

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been extensively studied in PMN stimulated with the chemotractant peptide fMLP. fMLP receptor signal transduction involves a pertussis toxin-sensitive G protein and activation of a phospholipase C, resulting in the production of inositol 1,4,5-trisphosphate (IP3) (16), which leads to the increase in 
\[Ca^{2+}\] (17). IP3 is thought to release Ca2+ directly from intracellular stores and indirectly open membrane calcium channels either through a metabolite or via increased \[Ca^{2+}\], itself (18–20).

Results presented in this report indicate that, in contrast to fMLP receptor stimulation, FcR ligation causes a \[Ca^{2+}\], rise via a pertussis toxin-insensitive pathway which is independent of IP3 generation. FcR ligation and fMLP binding apparently release Ca2+ to the cytosol from the same intracellular pool which is sensitive to the sesquieterpene lactone thapsigargin. Thus, signal transduction for increased \[Ca^{2+}\], in PMN initiated by different ligand-receptor interactions can occur by at least two distinct pathways.

**MATERIALS AND METHODS**

**Reagents**—A 10 times concentrated stock solution of Hanks’ balanced salt solution (HBSS) was obtained from Gibco. fMLP, phorbol myristate acetate, EDTA, and GTP were from Sigma. Antibodies of different specificities were from Biodesign International (Saco, ME). Alexa fluor dyes were obtained from Molecular Probes (Eugene, OR). 5% human albumin was from the New York Blood Center (New York, NY). RPMI-1640 tissue culture media was from Whittaker Bioproducts (Walkersville, MD). Fetal calf serum was from Hyclone (Logan, UT). Hybridoma cells producing anti-Fc receptor mAb were the kind gift of Dr. Jay Unkeless, Mt. Sinai Medical Center, New York, NY. 3G8 IgG was purified from ascites obtained from a B10.A(5R) mouse as described by Grynkiewicz et al. (24).

**Preparation of Neutrophil Suspensions**—PMN were obtained from heparinized venous blood from healthy adult donors. PMN were purified by standard techniques as previously described (22). Gener-
arly cells were suspended in HBBS, containing 10 mM HEPES, without Ca2+ or Mg2+. When necessary, 1.5 mM Ca2+ and 1.5 mM Mg2+ were added to this buffer (HBSS••).

**Aggregated IgG**—lG purified from human serum by octanoic acid precipitation (21) was heated at 60 °C for 30 min, chilled on ice, centrifuged at 12,000 rpm for 20 min, and chromatographed on a sizing column packed with Bio-Gel A-1.5 m, Bio-Rad. Aggregated IgG (agg-lgG) collected with the void volume was concentrated and stored at 4 °C.

**Insoluble Immune Complexes**—10 μl of bovine serum albumin (10 mg/ml) were mixed with 300 μl of rabbit anti-BSA antibody (2.6 mg/ml), incubated at 37 °C for 60 min, and then chilled on ice. The insoluble immune complexes formed were washed and resuspended in 300 μl of HBSS. A 25-fold final dilution of these preparations was used to stimulate FcR. This concentration was found to give in PMN a \[Ca^{2+}\], rise similar in magnitude to that elicited by 10−9 M fMLP.

**Fluorescence Calcium Measurements**—PMN were loaded with fura-2 AM as previously described (23). Fluorescence changes of a 2 ml-stirred PMN suspension kept at 37 °C were monitored with a F-2000 Hitachi Instruments (Danbury, CT) spectrofluorometer, using 340 and 380 nm excitation wavelengths and 510 nm emission wavelength. Calcium concentrations were calculated as described by Grynkiewicz et al. (24).

**Treatment of Neutrophils with Pertussis Toxin**—PMN at 10°6 cell/ml in HBSS•• were treated with pertussis toxin (PT) (0–10 μg/ml) at 37 °C for 75 min. After this time, cells were washed twice and resuspended in fresh buffer for [Ca2+] and IP3 measurements. 2 μg/ml pertussis toxin caused a complete inhibition of degradation, as measured by release of myeloperoxidase in response to fMLP (data not shown).

**Measurement of Inositol 1,4,5-Trisphosphate by RIA**—Neutrophils (3 × 106/ml) in buffer containing 20 mM LiCl were stimulated with 10−8 M fMLP or 300 μg/ml aggregated IgG. After various times an equal volume of 20% trichloroacetic acid was added. Cells were centrifuged and the supernatants were recovered, treated with 10 mM mannitol, and extracted four times with 3 ml of ether. The aqueous phase was neutralized with 7.5% NaHCO3 and used to measure IP3 with a commercial RIA kit following the manufacturer’s instructions.

**Separation of Inositol Phosphates by HPLC**—PMN were incubated in isotonic-free media with 5.5 mM glucose for 4 h in the presence of 50 μCi/ml [3H]inositol. Pertussis toxin (2 μg/ml) was added to the cells at the same time that the labeled inositol. Pertussis toxin did not affect the level of incorporation of radioactivity into the cells. 10 million PMN in HBSS•• containing 20 mM LiCl were treated with fMLP for 15 s, or agg-IgG or mAb 3G8 for 30 s. For these experiments the concentration of fMLP and agg-IgG was increased to 10−7 and 500 μg/ml, respectively, to induce maximum stimulation and to promote the highest production of inositol phosphates. Inositol phosphates were then isolated by trichloroacetic acid precipitation and ether extraction as described above. Samples were neutralized and applied to a S5 SAX Spherisorb HPLC column, from Phase Separations Inc. (Norwalk, CT). Inositol phosphates were eluted with an ammonium formate gradient from 0 to 1 M in 0.5 h. Reproducibility of the gradient was monitored by the resolution of a mixture of AMP, ADP, and ATP added as an internal standard to each sample. Fractions (0.5 ml) were collected and counted in 10 ml scintillation liquid. Peaks corresponding to inositol mono- (IP1), bi- (IP2), and trisphosphate (IP3) were identified by the retention time of known radioactive standards.

**RESULTS**

**[Ca2+]i Rise after FcR Ligation Is Pertussis Toxin Insensitive**—Neutrophils stimulated with 10−8 M fMLP showed a rapid increase in \[Ca^{2+}\], to approximately 280 nm above base line (which was 70 ± 18 nm; mean ± SD, n = 30). Ligation of Fc receptors by 300 μg/ml agg-IgG caused a \[Ca^{2+}\], rise to values around 130 nm over base line. This response to agg-


\begin{table}
| Buffer  | Increment of \([Ca^{2+}]\) | Increment of IP3 |
|---------|--------------------------|------------------|
| PT      | 0.79 ± 0.12             | 0.02 ± 0.16      |
| Buffer  | 0.35 ± 0.08             | ND               |
| nM      | 10                       | 35               |
| PMN     | 35 ± 11.1               | 0.16 ± 0.06      |
| FMLP (10 nM) | 0.20 ± 0.14            | ND               |
| 3G8     | 0.15 ± 0.06             | 0.60 ± 0.02      |
| 10G     | 0.27 ± 0.26             | 0.25 ± 0.11      |

*p < 0.01 compared to paired t test compared to PMN stimulated identically, but without PT pretreatment. IP3 measurements with the same time point were not statistically different from baseline.

ND, not done.
toxin for 60 min at
2 of the fMLP-induced 
not have any effect on agg-IgG-induced [Ca'\textsuperscript{2+}] rise (Fig. 3). Changes in cytosolic Ca'\textsuperscript{2+} concentration were assessed as in Fig. 1. 

IP\textsubscript{3} Is Not the Second Messenger for FcR-mediated [Ca'\textsuperscript{2+}] Rise—Since inositol 1,4,5-trisphosphate produced after stimulation of several types of receptors is known to mediate Ca'\textsuperscript{2+} release from intracellular stores (18, 19), we examined whether ligation of FcR and fMLP receptors also resulted in formation of IP\textsubscript{3}. PMN were stimulated with fMLP, agg-IgG, 3G8, or IIC for different periods of time and then inositol phosphates were isolated by trichloroacetic acid precipitation and ether extraction. IP\textsubscript{3} was quantitated in the neutralized cell extracts using an IP\textsubscript{3}-specific radioimmunoassay kit. Basal IP\textsubscript{3} measured in this way was 1.38 ± 0.24 pmol/10'\textsuperscript{6} PMN (mean ± S.E., n = 12). The relatively high levels of basal IP\textsubscript{3} measured by RIA, may reflect a partially activated state generated during the purification of PMN. fMLP induced an increase in formation of IP\textsubscript{3} by 15 s after stimulation (Table 1, Fig. 4). 1 nM fMLP produced a smaller but still significant and easily detectable peak of IP\textsubscript{3} with the same kinetics as 10 nM fMLP (Fig. 4). The effect of fMLP on both IP\textsubscript{3} and [Ca'\textsuperscript{2+}], was dose dependent, since 1 nM fMLP led to approximately 50% of the increases in [Ca'\textsuperscript{2+}], and IP\textsubscript{3} seen with 10 nM fMLP (Table 1, Fig. 4). Agg-IgG gave a [Ca'\textsuperscript{2+}] rise comparable in magnitude to that elicited by 1 nM fMLP (Table 1) but caused only very small IP\textsubscript{3} increases over base line (Fig. 4). In fact at no time was the measured IP\textsubscript{3} in response to either agg-IgG or 3G8 statistically different from base line. As expected, IP\textsubscript{3} formed after 10 nM fMLP stimulation was eliminated by pertussis toxin (Table 1). Importantly, the small amount of IP\textsubscript{3} generated by FcR ligation by either agg-IgG or 3G8 was not affected by pertussis toxin (Table 1). This lack of pertussis toxin effect on 3G8-induced [Ca'\textsuperscript{2+}], rise agrees with observations of others (14). Since the [Ca'\textsuperscript{2+}], increases observed after stimulation of FcR with agg-IgG and 3G8 were relatively small compared to the one coming after fMLP stimulation (Table 1), we examined the effect of stimulating PMN with insoluble BSA-anti BSA immune complexes (IIC). IIC gave a [Ca'\textsuperscript{2+}], rise in PMN of almost the same magnitude as the one elicited with 10\textsuperscript{\textup{-8}} M fMLP (Fig. 3, and Table 1). This [Ca'\textsuperscript{2+}], rise from IIC was more prolonged than the one from fMLP or agg-IgG.

Despite the marked increase in [Ca'\textsuperscript{2+}], rise compared to agg-IgG or 3G8, pertussis toxin treatment still had no effect on the response induced by IIC (Fig. 3). These results indicated that there is not a pertussis toxin-sensitive G protein involved in the mechanism of [Ca'\textsuperscript{2+}], release induced by FcR ligation in PMN. These differences in the generation of an increase in [Ca'\textsuperscript{2+}], by fMLP and FcR ligands led us to test the hypothesis that the molecular mechanisms involved in the increase were distinct for the two ligands.

**Fig. 1.** Fc receptor-induced [Ca'\textsuperscript{2+}], rise comes exclusively from intracellular stores. PMN were purified and loaded with fura-2 in HBSS\textsuperscript{c}, 5 × 10' PMN were stimulated with fMLP (10\textsuperscript{\textup{-8}} M) or aggregated IgG (300 \mu g/ml) or 40 \mu g/ml 3G8, in HBSS\textsuperscript{c} (calcium) or in 3 mM EGTA added to the buffer 1 min before stimulation. Changes in cytosolic Ca'\textsuperscript{2+} concentration were assessed by measuring the variations in fluorescence of fura-2. Values represent increment of [Ca'\textsuperscript{2+}], calculated by subtracting the base-line concentration from the maximal [Ca'\textsuperscript{2+}], reached after stimulation (mean ± S.E., n = 6).

**Fig. 2.** Pertussis toxin (PT) does not affect the [Ca'\textsuperscript{2+}], rise induced by aggregated IgG. PMN were treated with pertussis toxin for 60 min at 37°C and then stimulated with (A) fMLP (10\textsuperscript{\textup{-8}} M) or (B) aggregated IgG (300 \mu g/ml). Changes in cytosolic Ca'\textsuperscript{2+} concentration were assessed as in Fig. 1. Data are from one representative experiment of three experiments with similar results.

**Fig. 3.** Pertussis toxin (PT) does not affect the [Ca'\textsuperscript{2+}], rise induced by insoluble immune complexes. Control of pertussis toxin-treated neutrophils were stimulated with fMLP (10\textsuperscript{\textup{-8}} M) or BSA-anti-BSA-insoluble immune complexes prepared as described under "Materials and Methods." (25-fold dilution). Changes in cytosolic Ca'\textsuperscript{2+} concentration were assessed as in Fig. 1. [Ca'\textsuperscript{2+}], tracings are representative of three determinations with similar results.
pertussis toxin treatment (Table I). Treatment with pertussis toxin had no significant effect on the basal IP₃. Because pertussis toxin had no effect on FcR-dependent increase in [Ca²⁺], (Table I), these data suggested that the small rise in [Ca²⁺], caused by agg-IgG or 3G8 was unrelated to IP₃ generation.

To further compare FcR ligation with fMLP, IIC were used as an FcR ligand, at a concentration which gave an equivalent increase in [Ca²⁺], as 10⁻⁶ M fMLP (Fig. 3, Table I). Similar to agg-IgG and 3G8, the IP₃ produced after stimulation with IIC was minimal compared to fMLP and not statistically different from base line. The small nonsignificant rises in response to IIC were completely eliminated by pertussis toxin treatment (Table I). Kinetic experiments showed that there was no significant increase in IP₃ at any time from 5 to 30 s after IIC addition. These data demonstrate that even with an FcR ligand that gives equivalent [Ca²⁺] rise as 10 nm fMLP, no significant rise in IP₃, could be found. These data are consistent with the hypothesis that the increase in [Ca²⁺], caused by FcR ligation is unrelated to IP₃ generation.

To verify and extend the results obtained by RIA, we measured inositol phosphate turnover directly. In order to do this, PMN were labeled with [³H]inositol and treated with pertussis toxin or buffer for 75 min before stimulation. Cells were incubated with fMLP for 15 s and agg-IgG or 3G8 for 30 s, and then inositol phosphates were isolated and separated by HPLC. Pertussis toxin did not affect the level of incorporation of radioactivity into PMN or the base-line (unstimulated) levels of inositol phosphates. fMLP induced phosphoinositol turnover, manifested by the appearance of clear IP₁, IP₂, and IP₃ peaks above the nonstimulated level of inositol phosphates. Treatment with pertussis toxin completely prevented IP₁, IP₂, and IP₃ formation (Fig. 5). In agreement with the RIA, stimulation with agg-IgG caused a much smaller increase of inositol phosphates over nonstimulated conditions. In this assay, the amount of inositol phosphates generated by agg-IgG was statistically different from base line. Importantly, for all these inositol phosphates, this small rise was eliminated by pertussis toxin. Quantitatively similar results were obtained for mAb 3G8 stimulation, but the magnitude of the inositol phosphate accumulation was smaller (Fig. 5). These results confirmed the previous observations. These data supported the hypothesis that although a small amount of IP₃ can be detected after FcR stimulation, it is not coupled to the generation of the [Ca²⁺]; rise, since IP₃ is completely eliminated by treatment with pertussis toxin, but the increase in [Ca²⁺], is unaffected.

Signal Transduction by Fc Receptors

FIG. 4. FcR ligation causes minimal increases in IP₃. PMN were stimulated for various times with fMLP (10⁻⁷ M) (●), aggregated IgG (350 μg/ml) (●), mAb 3G8 (40 μg/ml) (▲), or fMLP (10⁻⁷ M) (○) and inositol phosphates isolated. IP₃ was measured by RIA. Values are mean ± S.E. of several determinations from n = 3 to n = 10 and represent increment of IP₃ over base line.

FIG. 5. Pertussis toxin (PT) inhibits release of inositol phosphates upon stimulation of either fMLP or Fc receptors. Neutrophils, labeled with [³H]inositol were left untreated (buffer) or treated with 2 μg/ml pertussis toxin before being stimulated with 10⁻⁷ M fMLP for 15 s, 500 μg/ml agg-IgG for 30 s, or 40 μg/ml 3G8 for 30 s. Inositol phosphates were then isolated and separated by HPLC. Values are mean ± S.E. of three experiments.

[Graphs and tables are not transcribed here]


Signal Transduction by Fc Receptors

10^8 M fMLP inhibited a subsequent increase in [Ca^{2+}], in response to thapsigargin (Fig. 6B). The maximum [Ca^{2+}] after thapsigargin in the absence of fMLP was 71.4 ± 2.1 nM (mean ± S.E., n = 5), while after fMLP, it was 24 ± 3.2 nM (mean ± S.E., n = 3), suggesting that ~69% of this intracellular pool had been depleted by prior exposure to fMLP. Thus, we concluded that 10^8 M fMLP effectively depleted this pool. Next, we stimulated PMN with IIC at a dose that gave a [Ca^{2+}], rise similar to that from 10^8 M fMLP. The [Ca^{2+}], rise after thapsigargin was almost completely eliminated when IIC were given before thapsigargin (Fig. 6B). The thapsigargin-induced [Ca^{2+}], rise was 25 ± 1.4 nM (mean ± S.E., n = 3) after IIC compared to 71.4 ± 2.1 nM (mean ± S.E., n = 5) when thapsigargin was given first, an extent of depletion similar to that seen with fMLP. Most significantly, IIC and fMLP were able to inhibit the [Ca^{2+}], rise due to subsequent stimulation with the other agonist (Figs. 6C and 7C). The maximum [Ca^{2+}], rise induced by IIC after fMLP was 69 ± 2.8 nM (mean ± S.E., n = 3) compared to 225 ± 25 nM (mean ± S.E., n = 8) in the absence of fMLP, a 69% inhibition, identical to that seen when thapsigargin was added after fMLP. Thus both fMLP and IIC could effectively deplete Ca^{2+} from a thapsigargin-sensitive pool, and each inhibited a subsequent [Ca^{2+}], rise from the other. We concluded that, despite the differences in mechanism of release of Ca^{2+} from intracellular stores, fMLP and Fc receptor ligation utilized the same intracellular Ca^{2+} pool. Interestingly, prior incubation with fMLP did not depress the [Ca^{2+}], rise induced by agg-IgG. In these experiments agg-IgG induced an increase in [Ca^{2+}], of 116 ± 8 nM (mean ± S.E., n = 8) when incubated with PMN prior to fMLP; agg-IgG induced an increase of 104 ± 2 nM when incubated after fMLP. Similarly, agg-IgG did not alter the fMLP-induced increase in [Ca^{2+}],. The fMLP-induced increase in [Ca^{2+}], was 303 ± 7 nM before agg-IgG and 291 ± 8 nM after agg-IgG. Nonetheless, prior treatment with thapsigargin eliminated the [Ca^{2+}], rise on subsequent exposure to agg-IgG. These data suggest that there was sufficient Ca^{2+} left in the thapsigargin-releasable pool after fMLP to support a normal submaximal FcR stimulation.

DISCUSSION

An increase in [Ca^{2+}], occurs as a result of interaction of IgG with PMN (29, 30). This increase in [Ca^{2+}], is thought to regulate multiple events which occur on ligation of Fc receptors, including degranulation (31), activation of arachidonate metabolism (32, 33), and diapedesis (34). Recently, we have shown that the increase in [Ca^{2+}], which takes place after Fc receptor ligation is necessary for fMLP induction of the highly phagocytic state characteristic of PMN at inflammatory sites (15). Therefore, we have investigated the mechanism by which [Ca^{2+}], is elevated after engagement of Fc receptors by model immune complexes.

Recent data from several laboratories have demonstrated that the IgG-mediated increase in [Ca^{2+}], comes entirely from release of intracellular stores of Ca^{2+} (14, 15). The best understood mechanism by which Ca^{2+} is released is by production of IP_3, which acts to open a channel in the membrane of the intracellular pool of Ca^{2+} (17, 35). IP_3 receptors have been purified and cloned from several tissues (36–41). These cDNAs predict a molecule with many of the characteristics expected of a ligand-gated channel. Therefore, we examined the hypothesis that the IgG-mediated increase in [Ca^{2+}], occurs because of generation of IP_3. Our data strongly suggest that this is not the case. First, although increases in [Ca^{2+}], in response to agg-IgG were about half of that achieved with an optimal concentration of fMLP, very little IP_3 gen-
eration could be detected from Fc receptor ligation, by either of two assays. Using insoluble immune complexes, which are a more physiological and potent stimulator for FcR, we found that the [Ca\(^{2+}\)] increase was as large as the one induced by fMLP. These immune complexes also generated very little IP\(_3\). Moreover, there was no clear relationship between the amount of IP\(_3\) generated and the [Ca\(^{2+}\)] increase induced by three different FcR ligands (3G8, agg-IgG, and IIC). In contrast, there was a correlation between IP\(_3\) generation and [Ca\(^{2+}\)], rise induced by thapsigargin, suggesting that fMLP had largely emptied this transduction mechanism for increase in [Ca\(^{2+}\)] by fMLP both the fMLP-induced IP\(_3\) accumulation and fMLP-induced concentration which was maximally effective in inhibition of Ca\(^{2+}\) release. Also in contrast to FcR ligation, the main signal receptors is via a pertussis toxin-sensitive Ca\(^{2+}\) release. Furthermore, under the same conditions of fMLP inhibited subsequent increase in [Ca\(^{2+}\)] caused by Ca\(^{2+}\) released by both fMLP (IP\(_3\)-dependent) and agg-IgG (IP\(_3\)-independent). Pretreatment of PMN with optimal doses of pertussis toxin abrogated. Therefore, there was a clear disparity between the effects of pertussis toxin on [Ca\(^{2+}\)] and IP\(_3\) after Fc receptor ligation. Similarly pertussis toxin had no effect on the [Ca\(^{2+}\)] rise induced by IIC. In contrast, pertussis toxin inhibited the increase in IP\(_3\) caused by agg-IgG, 3G8, and IIC, and indeed inhibited accumulation of all the water-soluble inositol phosphates observed with both fMLP and Fc receptor ligands. Thus, there was a clear disparity between the effects of pertussis toxin on [Ca\(^{2+}\)] and IP\(_3\) after fMLP ligation with agg-IgG and IIC. Normal increases in [Ca\(^{2+}\)] were observed in cells in which the IP\(_3\) response was completely abrogated.

In contrast, pertussis toxin treatment of PMN inhibited both the fMLP-induced IP\(_3\) accumulation and fMLP-induced [Ca\(^{2+}\)]. A very small [Ca\(^{2+}\)] increase (15% of normal) was still observed in maximally intoxicated cells, as reported by others (42), even though inositol phosphate turnover was not detected. This suggests that fMLP may also be able to release Ca\(^{2+}\) by a mechanism other than IP\(_3\). However, unlike Fc receptor ligation, this is a minor component of the signal for Ca\(^{2+}\) release. Also in contrast to FcR ligation, the main signal transduction mechanism for increase in [Ca\(^{2+}\)], by fMLP receptors is via a pertussis toxin-sensitive G protein pathway (16, 43).

To determine whether IgG induced release of Ca\(^{2+}\) from the same pool as fMLP, experiments were performed with thapsigargin. Thapsigargin has been used to distinguish between these immune complexes also generated very little IP\(_3\). The other IP\(_3\) insensitive and is activated by FcR stimulation.

The identity of the intracellular mediator of Ca\(^{2+}\) release induced by agg-IgG remains an important unsolved problem. It is notable that in other systems, fatty acids or their metabolites have been reported to release Ca\(^{2+}\) from an IP\(_3\)-sensitive pool, without inducing phosphoinositide turnover (45). While these experiments suggest a possible alternative intracellular Ca\(^{2+}\) ionophore, it is difficult in these in vitro experiments to exclude a detergent effect of the added fatty acids. In this regard our preliminary data show that low concentrations of para-bromophenacylbromide, a phospholipase A2 inhibitor, prevent the increase in Ca\(^{2+}\) in PMN after addition of agg-IgG but have little effect on fMLP-induced release. This may suggest that the phospholipase A2 activity, which is normally associated with some forms of IgG Fc receptor (46, 47), is necessary for the increase in [Ca\(^{2+}\)], which follows recognition of both particulate and soluble immune complexes.

REFERENCES

1. Unkeless, J. C., Sciglione, E., and Freedman, V. H. (1988) Annu. Rev. Immunol. 6, 251–281
2. Kinet, J.-P. (1989) Cell 57, 351–354
3. Unkeless, J. C., and Wright, S. D. (1988) In Inflammation: Basic Principles and Clinical Correlates (Gallin, J. I., Goldstein, I. M., and Snyderman, R., eds) pp. 343–362, Raven Press, Ltd., New York.
4. Anderson, C. L., and Looney, R. J. (1986) Immunol. Today 7, 264–266
5. Stuart, S. G., Trounstine, M. L., Vaux, D. J. T., Koch, T., Martens, C. L., Mellow, I., and Moore, K. W. (1987) J. Exp. Med. 166, 1588–1594
6. Hibbs, M. L., Bonadonna, L., Scott, B. M., McKenzie, I. F. C., and Hogarth, P. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2240–2244
7. Huizinga, T. W., van der Schout, C. E., Jost, C., Klassen, R., Kleijer, M., von dem Borne, A. E. G. K., Roos, D., and Tettero, P. A. T. (1988) Nature 333, 667–669
8. Laniar, L. L., Ruitenmeer, J. J., and Phillips, J. H. (1988) J. Immunol. 141, 3478–3485
9. Mellow, I. (1988) Curr. Opin. Immunol. 1, 16–25
10. Unkeless, J. C. (1989) Curr. Opin. Immunol. 2, 63–67
11. Huizinga, T. W., Van Kemenade, F., Koenderman, L., Dolman, K. M., Von Der Borne, A. E. G. K., Tetteroo, P. A. T., and Roos, D. (1989) J. Immunol. 142, 2365–2369
12. Anderson, C. L., Shen, L., Eicher, D. M., Wewers, M. D., and Gill, J. K. (1990) J. Exp. Med. 171, 1335–1345
13. Huizinga, T. W., Dolman, K. M., Van Der Linden, N. J. M., Kleijer, M., Nijenhuis, J. H., Von Dem Borne, A. E. G. K., and Roos, D. (1990) J. Immunol. 144, 1432–1437
14. Kimberly, R. P., Ablstrom, J. W., Click, M. E., and Edberg, J. C. (1990) J. Exp. Med. 171, 1239–1255
15. Rosales, C., and Brown, E. J. (1991) J. Immunol. 146, 3937–3944
16. Snyderman, R., and Uehling, R. J. (1988) In Inflammation: Basic Principles and Clinical Correlates (Gallin, J. I., Goldstein, I. M., and Snyderman, R., eds) pp. 309–323, Raven Press, Ltd., New York.
17. Freuiti, M., Wollheim, C. G., and Lew, P. D. (1984) J. Biol. Chem. 259, 13777–13782
18. Berridge, M. J., and Irvine, R. F. (1989) Nature 341, 197–205
19. Putney, J. W., Jr., Takeamura, H., Hughes, A. E., Horstman, D. A., and Thastrup, O. (1989) FASEB J. 3, 1899–1905
20. Irvine, R. F., and Moor, R. M. (1988) Biochem. J. 240, 917–920
21. Hurn, B. A., and Chantler, S. M. (1980) Methods Enzymol. 74, 104–142
22. Gresham, H. D., Clement, L. T., Volanakis, J. E., and Brown, E. J. (1987) J. Immunol. 139, 4159–4166
23. Korshak, H. M., Vossall, L. B., Ljubich, P., Rich, A. M., and Weissmann, G. (1988) J. Biol. Chem. 263, 11090–11097
24. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
25. Mertit, J. E., Jacob, R., and Hallam, T. J. (1989) J. Biol. Chem. 264, 1522–1527
26. Ghosh, T. K., Mullaney, J. M., Tarazi, F. I., and Gill, D. L. (1989) Nature 340, 236–239
27. Meldolesi, J., Madeddu, L., and Pozzan, T. (1990) Biochim. Biophys. Acta 1065, 130–140
28. Thastrup, O., Cullen, P. J., Dråbak, B. K., Hanley, M. R., and Dawson, A. P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2466-2470
29. Young, J. D., Ko, S. S., and Cohn, Z. A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 5430-5434
30. Lew, D. P., Anderson, T., Hed, J., Di Virgilio, F., Pozzan, T., and Stendahl, O. (1985) Nature 315, 509-511
31. Perez, D. H., Marder, S., Elfman, F., and Ives, H. (1987) Biochem. Biophys. Res. Commun. 145, 976-981
32. Kadiri, C., Masliah, J., Bachelet, M., Vargaftig, B. B., and Béreziat, G. (1989) J. Cell Biochem. 40, 157-164
33. Pawlowski, N. A., Kaplan, G., Hamil, A. L., Cohn, A. L., and Scott, W. A. (1983) J. Exp. Med. 158, 393-412
34. Marks, P. W., and Maxfield, F. R. (1990) J. Cell Biol. 110, 43-52
35. Berridge, M. J. (1987) Annu. Rev. Biochem. 56, 159-193
36. Maeda, N., Niinobe, M., and Mikoshiba, K. (1990) EMBO J. 9, 61-67
37. Supattapone, S., Worley, P., Baraban, J. M., and Snyder, S. H. (1988) J. Biol. Chem. 263, 1530-1534
38. Chadwick, C. C., Saito, A., and Fleiacher, S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2132-2136
39. Ferris, C. D., Huganir, R. L., Supattapone, S., and Snyder, S. H. (1989) Nature 342, 87-89
40. Furutchi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., and Mikoshiba, K. (1989) Nature 342, 32-38
41. Marks, A. R., Tempst, P., Chadwick, C. C., Riviere, L., Fleisher, S., and Nadal-Ginard, B. (1990) J. Biol. Chem. 265, 20719-20722
42. Rossi, F., Della Bianca, V., Grzeskowiak, M., De Togni, P., and Gahrim, G. (1985) FEBS Lett. 181, 253-258
43. Smith, C. D., Lane, B. C., Kusaka, I., Verghese, M. W., and Snyderman, R. (1985) J. Biol. Chem. 260, 5875-5878
44. Takekuma, H., Hughes, A. R., Thastrup, O., and Putney, J. W., Jr. (1989) J. Biol. Chem. 264, 12266-12271
45. Chow, S. C., and Jondal, M. (1990) J. Biol. Chem. 265, 902-907
46. Suzuki, T., Saito-Taki, T., Sadasivan, R., and Ninna, T. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 591-595
47. Nitta, T., Saito-Taki, T., and Suzuki, T. (1984) J. Leukocyte Biol. 36, 493-504