Calcium-G Protein Interactions in the Regulation of Macrophage Secretion*

Received for publication, June 1, 2001, and in revised form, July 6, 2001
Published, JBC Papers in Press, July 30, 2001, DOI 10.1074/jbc.M105038200

Anke Di, Boris Krupa, and Deborah J. Nelson‡

From the Department of Neurobiology, Pharmacology and Physiology, The University of Chicago, Chicago, Illinois 60637

The interplay between activated G proteins and intracellular calcium ([Ca\(^{2+}\)]\(_i\)) in the regulation of secretion was studied in the macrophage, coupling membrane capacitance with calcium-sensitive microfluorimetry. Intracellular elevation of either the nonhydrolyzable analogue of GTP, guanosine-5’-O-(3-thiotriphosphate) (GTP\(_\gamma\)S), or [Ca\(^{2+}\)]\(_i\), enhanced the amplitude and shortened the time course of stimulus-induced secretion in a dose-dependent manner. Both the ionophore- and the stimulus-induced secretory response were abolished in the presence of guanosine-5’-O-(2-thiodiphosphate) (GDP\(_\beta\)S). The \(K_d\) of Ca\(^{2+}\)-driven secretion was independent of GTP\(_\gamma\)S concentration, whereas the \(K_d\) of the GTP\(_\gamma\)S-driven response decreased from 63 to 31 \(\mu\)M in the presence of saturating concentrations of [Ca\(^{2+}\)]\(_i\). The time course of stimulus-induced secretion was dependent upon the concentration of [Ca\(^{2+}\)]\(_i\). The time course of GTP\(_\gamma\)S-driven secretion was concentration-independent at high levels of [Ca\(^{2+}\)]\(_i\)], suggesting that a calcium-dependent translocation/binding step was rate-limiting. Our data strongly support a model in which [Ca\(^{2+}\)]\(_i\) and activated G proteins act independently of one another in the sequential regulation of macropage secretion. [Ca\(^{2+}\)]\(_i\) appears to play a role in the recruitment and priming of vesicles from reserve intracellular pools at a step that is upstream of G protein activation. While activated, G proteins appear to play a key role in fusion of docked vesicles. Thus, secretion can result either from activating more G proteins or from elevating [Ca\(^{2+}\)]\(_i\) at basal levels of G protein activation.

The class of phagocytic inflammatory cells encompasses the mononuclear phagocytes, including circulating monocytes, tissue macrophages, neutrophils, and eosinophils. The macrophage is the primary differentiated cell of this system and plays a major role in host defense against microbial infections. Macrophages possess a diversity of plasma membrane receptors that recognize and bind both particulate and soluble stimuli found in body fluids. In addition, hematopoietic cells in general and macrophages in particular contain several distinct secretory organelles that appear to be selectively mobilized for secretion by different stimuli. In macrophages this may include different types of dense core granules as well as phagosomes and phagolyososomes. Although the initial steps in the intracellular signal transduction cascades from the receptors that eventuate secretion and/or phagocytosis are known, the Ca\(^{2+}\) and G protein dependence of the pathway(s) that links receptor binding and cross-linking to vesicle fusion is still unclear.

In the studies described in this investigation, heat-aggregated immunoglobulin G (HAIGG), which binds to and aggregates Fc receptors, was used to mimic immune complex stimulation. When Fc-Fc receptors are engaged at the cell surface by the opsonized surface of interacting particles, the resultant receptor clustering initiates tyrosine phosphorylation of cytoplasmic residues in the immunoreceptor tyrosine activation motifs by Src-family kinases. The resultant tyrosine-phosphorylated residues form anchoring sites for SH2 domain-containing proteins, the most important of which seems to be the non-receptor tyrosine kinase Syk, which can phosphorylate several downstream substrates involved in the phagocytic response. One such substrate is the Rho family guanine nucleotide exchange factor Vav, which activates the small G proteins Rac and Cdc42. HAIGG, thus, was used in our studies to maximize G protein turnover contributing to actin polymerization and, as our data demonstrate, the exocytotic fusion of vesicles. The studies were carried out to elucidate the relative roles of intracellular calcium ([Ca\(^{2+}\)]\(_i\)) and activated G proteins in mediating the secretory response in the activated macrophage.

Membrane capacitance is proportional to cell surface area, and therefore, the measurement of membrane capacitance has become an important technique for studying exocytosis and endocytosis in a wide variety of secretory cells (1–11). Macrophage stimulation is accompanied by a complex series of capacitance changes reflective of exocytosis and phagocytosis (12). In this study we have taken advantage of the differential sensitivity of phagocytosis and exocytosis to temperature and [Ca\(^{2+}\)]\(_i\), to study the regulation of the stimulus-induced secretory response in isolation.

Classically, in cells of neurosecretory origin, regulated secretory responses are triggered by an increase in the [Ca\(^{2+}\)]\(_i\) through the opening of voltage-activated Ca\(^{2+}\) channels. GTP-binding proteins clearly play a role, but they appear to be subservient to the primary Ca\(^{2+}\) signal (very little secretion occurs with GTP\(_\gamma\)S at resting [Ca\(^{2+}\)]\(_i\), levels). By contrast, the regulated secretory response of hematopoietic cells is substantially dependent on G proteins, whereas Ca\(^{2+}\) seems to play a more modulatory role (6, 13, 14). Studies in permeabilized mast cells, neutrophils, and eosinophils show that secretion can be induced by GTP\(_\gamma\)S in the effective absence of [Ca\(^{2+}\)]\(_i\), (<10\(^{-3}\) m) (15). In contrast to mast cells, very little is known about the regulation of secretion in phagocytes, and our studies represent

* This work was supported by NIGMS, National Institutes of Health Grant R01 GM36823. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: University of Chicago, Dept. of Neurobiology, Pharmacology, and Physiology, 947 E. 58th St., MC 0926, Chicago, IL 60637. Tel.: 773-702-0126; Fax: 773-834-4522; E-mail: dnelson@drugs.bsd.uchicago.edu.

1 The abbreviations used are: HAIGG, heat-aggregated immunoglobulin G; [Ca\(^{2+}\)]\(_i\), intracellular calcium; GDP\(_\beta\)S, guanosine-5’-O-2-thiodiphosphate; GTP\(_\gamma\)S, guanosine-5’-O-(3-thiotriphosphate); SNARE, soluble N-ethylmaleimide factor attachment protein receptor; GTP, endogenous GTP; pF, picofarads.
a different approach to this problem.

Previous studies suggested that the regulated secretory response of the phagocyte, as with other cells in the immune system, are triggered by guanine nucleotides and shaped in time course by local gradients in \( \text{[Ca}^{2+}] \), (6, 13, 14). However, the interplay between the two signaling elements has made it difficult to study their individual effects, since in both mast cells and neutrophils, GTP-\( \Psi \)S induces a transient increase in \( \text{[Ca}^{2+}] \), in cells that are weakly \( \text{Ca}^{2+} \)-buffered (6, 13, 16) by a mechanism that is still unknown. Therefore, to elucidate the role of \( \text{[Ca}^{2+}] \), and GTP-binding proteins in the signal transduction cascade, we studied their effects on secretion in isolation. Cells were stimulated through three pathways; they are 1) surface Fe receptor ligation by multivalent ligands that enhanced the turnover of G proteins in the presence of elevated \( \text{[Ca}^{2+}] \), 2) exposure to the \( \text{Ca}^{2+} \) ionophore A23187 in \( \text{Ca}^{2+} \)-containing external solutions in the absence of enhanced G protein activation, and 3) intracellular application of GTP-\( \Psi \)S in highly \( \text{Ca}^{2+} \)-buffered internal solutions with and without receptor stimulation. Previous data obtained from the mast cell indicated that \( \text{[Ca}^{2+}] \), influenced the latency and rise time but not the amplitude of the degradation response (6). In our studies, both the time course and amplitude of secretion in the macrophage is a sensitive function of \( \text{[Ca}^{2+}] \), suggesting that \( \text{Ca}^{2+} \)-mediated regulatory control of secretion is present at least in some cells of hematopoietic origin. Our data suggest that, like the mast cell, \( \text{Ca}^{2+} \) may be permissive and GTP may be essential to the secretory process. Thus, GTP-\( \Psi \)S can elicit a capacitance increase in the nominal absence of \( \text{Ca}^{2+} \), and \( \text{Ca}^{2+} \)-induced secretion is ablated by GDP-\( \Psi \)S (a GDP analogue that prevents G proteins from binding GTP). Moreover, the presence of GTP-\( \Psi \)S does not alter the \( \text{Ca}^{2+} \) sensitivity of secretion, whereas the \( K_d \) for GTP-\( \Psi \)S is reduced in the presence of optimal \([\text{Ca}^{2+}] \).

It has become clear that the final stages of exocytosis in cells of hematopoietic origin are determined by the activation of GTP-binding proteins. The nature of the specific G proteins involved in hematopoietic cell secretion is, however, still not entirely clear. Early investigators proposed the existence of an elusive \( G \) protein, implying that it was hetrotrimeric in nature (17, 18). However, there is a growing body of evidence that one or more of the small GTP-binding proteins, including members of the Rho family (Rho, Rac, Cdc42) as well as Ras, Rab, and ARF family members may be involved in vesicle mobilization, docking, and/or membrane fusion in cells of hematopoietic origin (15, 19–22). For example, antagonism of Rho family function using specific proteins appears to block mast cell degranulation in a permeabilized cell model (20, 23). Rab GTPases appear to recruit tethering and docking proteins from the cytosol to the membrane after an interconversion between inactive, GDP-bound forms and active, GTP-bound forms, a process that does not appear to require GTP hydrolysis (22). Rab not only appears to regulate vesicle docking but also has been found to enable SNARE complex formation preceding and, finally, permitting vesicle fusion (for review, see Ref. 22). Consistent with these findings, our data suggest a role for a GTPase-dependent step in the final stages of vesicle fusion.

The rationale for the present studies was the unresolved role that activated G proteins and \( \text{Ca}^{2+} \) play in the regulation of exocytosis in the macrophage. The controversy resides in \( \text{Ca}^{2+} \) versus the GTP sensitivity of the response. The early studies of Neher (6) on the mast cell suggest that elevations in \( \text{[Ca}^{2+}] \), do not lead to secretion in the absence of GTP-\( \Psi \)S. Instead it was proposed that GTP-\( \Psi \)S was involved in priming the \( \text{Ca}^{2+} \)-regulated exocytotic response. Although \( \text{Ca}^{2+} \) did not initiate secretion, the response was interpreted as ultimately \( \text{Ca}^{2+} \)-regulated in that increases in \( \text{[Ca}^{2+}] \), accelerated the time course of degranulation. However, the dose dependence of secretion on the levels of activated G proteins was not investigated. Our studies were carried out under conditions in which we examined the sensitivity of the G protein regulatory arm of the response in isolation from the \( \text{Ca}^{2+} \)-dependent arm. We demonstrate that the pathway linking receptor binding and exocytosis induced by human HAIGG involves both G protein activation and calcium signaling as independent yet synergistic secretory stimuli. Although activated G proteins are both necessary and sufficient stimuli for secretion, the response in the macrophage is highly sensitive to changes in \( \text{[Ca}^{2+}] \), both in amplitude and time course. The fact that GTP-\( \Psi \)S increases the amplitude but not the sensitivity of \( \text{Ca}^{2+} \)-driven exocytosis suggests that the \( \text{Ca}^{2+} \) regulatory step is independent and upstream of the G protein-sensitive step and that the two regulatory pathways are additive. The observation that \( \text{Ca}^{2+} \) shifts the sensitivity of GTP-\( \Psi \)S-induced secretion indicates that the activated G protein-sensitive step is downstream of the \( \text{Ca}^{2+} \) regulatory step, and its sensitivity can be enhanced by \( \text{Ca}^{2+} \) priming. Therefore, unlike secretion in the mast cell, where GTP increases the \( \text{Ca}^{2+} \) sensitivity of exocytosis (6) and G proteins are thought to prime a \( \text{Ca}^{2+} \) regulated response, in our studies of stimulus-induced secretion in the macrophage, \( \text{Ca}^{2+} \) enhances the sensitivity of a GTP-dependent process.

MATERIALS AND METHODS

Cell Culture—J774.1 cells were obtained from ATCC and were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were plated on 35-mm tissue culture dishes for up to 1–2 days before the electrophysiological experiments.

Electrophysiology—Whole-cell recordings of J774.1 cells were obtained using the techniques of Hamill et al. (24). Pipettes were obtained using quartz glass (Sutter Instrument Co., Novato, CA) and a Sutter model P-2000 puller and had resistances of ~6–8 megohms.

Coupled Microfluorimetry and Electrophysiology—Simultaneous fluorometric and capacitance measurements were recorded in single cells as previously described (25). Briefly, a Leitz DM IRB (Leica, Germany) inverted microscope was illuminated using a Photon Technology International Nordic us (Monmouth Junction, NJ) dual monochromator photomultiplier tube equipped with a xenon lamp and a photomultiplier. Cells were loaded with a 100 \( \mu \)M fura-2 penta-potassium by diffusion from the recording pipette. Once the dye reached a maximum level within the cell, simultaneous measurements of capacitance and fura-2 fluorescence at 340 and 380 nm were acquired using an EPC-9 computer-controlled patch clamp amplifier (HEKA Electronic, Lambrecht, Germany) running PULSE software (HEKA). The EPC-9 includes a built-in data acquisition interface (ITC-16, Instrutech, NY). The software package controlled the stimulus and data acquisition for the software lock-in amplifier in the sine + dc mode, as described by Gillis (26). The holding potential throughout the experiments was –10 mV. The temporal resolution of the capacitance data was 40 ms/point using a 1-kHz, 20-mV sine wave. The PULSE software simultaneously controlled the acquisition of the electrophysiological and fluorescence data from the voltage-clamped cell. The temporal resolution of the fluorescence data was 20 ms/point. Data were acquired using a Pentium 3 PC and analyzed offline using the integrated graphics package, IGOR Pro (WaveMetrics, Lake Oswego, OR).

Calibration of Cellular fura-2 Signals—All calibrations were performed with dye-loaded cells according to the patch-pipette loading method of Almers and Neher (27). Cells were loaded with dye via the patch clamp pipette. The pipette solution contained a weakly buffered solution of \( \text{K} \) a and a photomultiplier. Cells were loaded with a 100 \( \mu \)M fura-2 penta-potassium by diffusion from the recording pipette. Once the dye reached a maximum level within the cell, simultaneous measurements of capacitance and fura-2 fluorescence at 340 and 380 nm were acquired using an EPC-9 computer-controlled patch clamp amplifier (HEKA Electronic, Lambrecht, Germany) running PULSE software (HEKA). The EPC-9 includes a built-in data acquisition interface (ITC-16, Instrutech, NY). The software package controlled the stimulus and data acquisition for the software lock-in amplifier in the sine + dc mode, as described by Gillis (26). The holding potential throughout the experiments was –10 mV. The temporal resolution of the capacitance data was 40 ms/point using a 1-kHz, 20-mV sine wave. The PULSE software simultaneously controlled the acquisition of the electrophysiological and fluorescence data from the voltage-clamped cell. The temporal resolution of the fluorescence data was 20 ms/point. Data were acquired using a Pentium 3 PC and analyzed offline using the integrated graphics package, IGOR Pro (WaveMetrics, Lake Oswego, OR).

Calibration of Cellular fura-2 Signals—All calibrations were performed with dye-loaded cells according to the patch-pipette loading method of Almers and Neher (27). Cells were loaded with dye via the patch clamp pipette. The pipette solution contained a weakly buffered solution of \( \text{K} \) a. Once the ratio at the known resting \( \text{[Ca}^{2+}] \), was determined the cell was stimulated with HAIGG, and the resultant 340/380 ratio was determined. Each data point at the known \( \text{[Ca}^{2+}] \), was averaged for 3–5 cells. \( \text{[Ca}^{2+}] \), was estimated for the HAIGG-stimulated cells by fitting the known \( \text{[Ca}^{2+}] \), data with the equation \( \text{[Ca}^{2+}] = K_0 \cdot A_{\text{max}} \cdot (R - R_{\text{min}}) / (K - R - R_{\text{min}}) \), where \( K \) was estimated from the data, \( R_{\text{min}} \) was determined with a pipette solution containing 10 mM BPATC and no \( \text{Ca}^{2+} \), and \( R_{\text{max}} \) was determined with a pipette solution containing 2 mM \( \text{Ca}^{2+} \).

Solutions—Experiments were carried out using a standard pipette solution that contained 80 mM potassium aspartate, 40 mM KCl, 2 mM MgCl₂, 10 mM HEPES buffered to pH 7.2, and a Ca-EGTA buffer to yield a
Fig. 1. Time course of changes in membrane capacitance after exposure of J774.1 cells to HAIGG as a function of temperature and intracellular calcium. Changes in membrane capacitance are shown for four different cells under the recording conditions indicated by the symbols on the top of each capacitance trace. All cells were exposed to the extracellular stimulus HAIGG (100 μg/ml) as indicated by the arrow. All recordings were made at a holding potential of −10 mV. A, recording demonstrating the biphasic nature of the response in a voltage-clamped cell exposed to HAIGG when the internal free Ca\(^{2+}\) was buffered to 0 mM Ca\(^{2+}\) (1 mM EGTA and 0.2 mM Ca\(^{2+}\)) in the pipette solution; the bath solution contained 2 mM Ca\(^{2+}\). The complex response consisted of a rapid secretory phase that was followed by a rapid uptake phase which showed stepwise decreases corresponding to the formation of individual phagocytic vesicles. Note that the peak of the biphasic response induced by HAIGG in the presence of high levels of external and internal Ca\(^{2+}\) is an under-estimate of the magnitude of the change, since it is likely that both exocytosis and phagocytosis occur simultaneously. B, recording at vanishingly low levels of internal free Ca\(^{2+}\) with internal solutions containing 10 mM BAPTA and nominally Ca\(^{2+}\)-free external solution; under these conditions, the response to the particulate stimulus was monophasic and consisted only of a secretory phase. C, recording made under the same conditions as in A but at lower temperature, as indicated. Note the lack of phagocytic response at this temperature. D, recording demonstrating the same response as in B but at a lower temperature. Note the increase in the time course and reduction in the amplitude of exocytic response at the lower temperature. Recording conditions are identical in all figures unless otherwise indicated. The pipette solution contained 80 mM potassium aspartate, 40 mM KCl, 1 mM EGTA, 3 mM KOH, 2 mM MgCl\(_2\), 10 mM HEPES buffered to pH 7.4; 2 mM Ca\(^{2+}\) was added as indicated by the symbol.

Desired free Ca\(^{2+}\) concentration. The CaCl\(_2\) and EGTA composition of the internal solutions varied according to experimental protocol. The 40, 60, 100, and 130 mM free Ca\(^{2+}\) pipette solution was obtained by adding 1.1 EGTA mixed with 0.2, 0.3, 0.4, and 0.5 CaCl\(_2\), respectively, to the standard pipette solution described above. The free Ca\(^{2+}\) concentration was calculated assuming an apparent dissociation constant of the Ca-EGTA complex of 0.15 μM at pH 7.2 using the methodology of Fabiato and Fabiato (28). In experiments where intracellular Ca\(^{2+}\) was required to be 0, 10 mM BAPTA was added to the extracellular solution. The bath solution contained 100 mM NaCl, 50 mM KCl, 2 mM MgCl\(_2\), 2 mM CaCl\(_2\), and 10 mM HEPES, pH 7.4. CaCl\(_2\) was excluded from the bath solution when intracellular Ca\(^{2+}\) was required to be 0. The theoretical equilibrium potentials for K\(^+\) and Cl\(^-\) using these solutions were \(E_K = -21\) mV and \(E_{Cl} = -31\) mV. Solution osmolalities were measured with a vapor pressure osmometer (Model 5500, Wescor, Logan, UT) and measured to be 290 mosmol.

Materials—Human IgG was obtained from Accurate Chemical (Westbury, NY). GTP\(_S\) and GDP\(_S\) were obtained from Sigma. HAIGG was prepared as previously described (29).

Data Analysis—Summary data are expressed as means ± S.E., with the number of experiments in parentheses. Figs. to the data were performed using the data analysis and technical graphics software package Origin (Microcal Software, Inc., Northhampton, MA). The error given in the text for the \(K_p\) is derived from the non-linear fit to the data and corresponds to \(σ_n = (C_mx^2)^{1/2}\), where \(C_m\) is the diagonal element of the variance-covariance matrix.

RESULTS

Temperature and Ca\(^{2+}\) Dependence of Phagocytosis—The secretory response in the cultured macrophage was assayed using measurements of membrane capacitance (\(C_m\)) in cells of the murine macrophage-like cell line, J774.1. The time course of changes in \(C_m\) in response to Fc receptor binding and crosslinking by HAIGG can be seen in Fig. 1. When intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) was weakly buffered with an internal solution containing 1.1 mM EGTA and 0.2 mM Ca\(^{2+}\), the cellular response to HAIGG added to the Ca\(^{2+}\)-containing bath solution (2 mM Ca\(^{2+}\)) was biphasic at 37 °C, as seen in Fig. 1A and summarized in Fig. 2A. The response consisted of an initial rapid increase in \(C_m\) (2.1 ± 0.7 pF (n = 10)) followed by a pronounced decrease in \(C_m\) (4.4 ± 0.2 pF (n = 10)) corresponding to particle uptake or phagocytosis. If [Ca\(^{2+}\)]\(_i\) was buffered with 10 mM BAPTA and the bath solution was nominally Ca\(^{2+}\)-free, the phagocytic response to HAIGG was absent, leaving the exocytic response in isolation (6.9 ± 1.2 (n = 6)), as seen in Fig. 1B and summarized in Fig. 2A. The phagocytic phase was inhibited at lower temperatures (25 °C) even when [Ca\(^{2+}\)]\(_i\) was weakly buffered (1.1 mM EGTA and 0.2 mM Ca\(^{2+}\)). Although secretion still occurred at room temperature, its time course and amplitude were both temperature and Ca\(^{2+}\)-dependent (see Fig. 1, B, C, and D, and Fig. 2). We took advantage of the temperature sensitivity of the phagocytic response to study the regulation of the secretory response in isolation.

Temperature Dependence of Secretion—Both the time course and amplitude of the secretory response induced by HAIGG were temperature-dependent as seen in Fig. 1, B and D, and summarized in Fig. 2. A decrease in temperature from 37 to 25 °C with BAPTA-containing internal solutions resulted in a decrease in the magnitude of secretion from 6.9 ± 1.2 pF (n = 6) to 0.7 ± 0.2 pF (n = 3). The time to half-maximal response under the same conditions increased from 20.8 ± 6.9 s (n = 6) to 50.3 ± 0.8 s (n = 3). One interpretation of these results is that exocytosis is contaminated with endocytosis at 37 °C with 40 mM [Ca\(^{2+}\)]\(_i\), thus giving rise to an attenuated secretory response. This would account for the difference in exocytosis observed between 37 and 25 °C at the elevated [Ca\(^{2+}\)]\(_i\).

G Proteins Are Essential for Exocytosis—Secretion in mast cells (6, 14), neutrophils (13), and eosinophils (30) is triggered by the nonhydrolyzable analogue of GTP, GTP\(_S\). GTP\(_S\) has been shown to evoke transient increases in [Ca\(^{2+}\)]\(_i\), in both mast
cells and neutrophils (6, 16). We examined the interplay between guanine nucleotides and [Ca\textsuperscript{2+}], in the regulation of exocytosis in macrophages by intracellular application of weakly and highly buffered Ca\textsuperscript{2+} internal solutions in the presence and absence of GTP\textgamma;S as well as the inhibitory analogue, GDP\beta;S. Cells were stimulated with HAIGG to elicit the secretory response, and [Ca\textsuperscript{2+}], and C\textsubscript{m} were simultaneously monitored with fura-2 (100 \mu M), loaded via the patch pipette. As seen in Fig. 3A, when intracellular Ca\textsuperscript{2+} was highly buffered with 10 mM BAPTA, HAIGG failed to induce an increase in [Ca\textsuperscript{2+}], but still triggered exocytosis (0.8 ± 0.4 pF (n = 5)). When [Ca\textsuperscript{2+}], was weakly buffered to 100 nM (1.1 mM EGTA and 0.4 mM Ca\textsuperscript{2+}) as in Fig. 3B, HAIGG triggered an increase in [Ca\textsuperscript{2+}], and C\textsubscript{m} (6.1 ± 0.4 pF (n = 5)). Secretion was inhibited when the BAPTA-containing internal solutions included GDP\beta;S (1 mM) (Fig. 3C). And finally, although the Ca\textsuperscript{2+} transient was preserved when [Ca\textsuperscript{2+}], was weakly buffered to 100 nM, intracellular GDP\beta;S (1 mM) inhibited the HAIGG-induced increase in C\textsubscript{m} (Fig. 3D). Thus, it appears that G protein activation is essential for a full exocytic response.

**Elevation of [Ca\textsuperscript{2+}], as well as GTP\gamma;S Are Sufficient to Induce Exocytosis in the Absence of an External Stimulus**—We next addressed the question of whether Ca\textsuperscript{2+} is a sufficient signal for secretion with other GTP-sensitive molecules merely acting as supportive components of the response. As in the previous set of experiments, both C\textsubscript{m} and [Ca\textsuperscript{2+}], were simultaneously recorded in single cells. Cells were stimulated with the Ca\textsuperscript{2+} ionophore A23187 (10 \mu M), added to the bath solution to bypass receptor-mediated events in the presence or absence of intracellular GDP\beta;S to block activation of G proteins. Alternatively, cells were dialyzed with high concentrations of GTP\gamma;S to render G proteins maximally active under conditions in which internal [Ca\textsuperscript{2+}], was buffered to varying levels as assayed by changes in the fura-2 signal. Sample data obtained in these four conditions are given in Fig. 4, A–D, and summarized in Fig. 4, E and F. The ionophore A23187 induced a large increase in capacitance (3.5 ± 0.8 pF (n = 6)) as well as a rapid increase in the level of [Ca\textsuperscript{2+}], when intracellular Ca\textsuperscript{2+} was weakly buffered (Fig. 4A). Adding GDP\beta;S (1 mM) to the internal solution completely inhibited the capacitance increase, but not the [Ca\textsuperscript{2+}], level, in response to the ionophore, demonstrating that Ca\textsuperscript{2+} induced exocytosis is extremely dependent on activated G proteins (Fig. 4B). Intracellular application of 400 \mu M GTP\gamma;S alone evoked a secretory response (3.2 ± 0.5 pF (n = 12)) as well as a rapid increase in the level of [Ca\textsuperscript{2+}], when the internal solution was weakly buffered (Fig. 4C). The secretory response to GTP\gamma;S was not significantly lower in amplitude than that induced by the Ca\textsuperscript{2+} ionophore in the presence of endogenous levels of GTP (3.5 ± 0.8 pF (n = 6)). GTP\gamma;S-induced secretion was still remained but with a significantly reduced amplitude (1.1 ± 0.3 pF, (n = 4)) when [Ca\textsuperscript{2+}], was highly buffered by 10 mM BAPTA (Fig. 4, D and F).

[Ca\textsuperscript{2+}], Increases the Sensitivity of Exocytosis Induced by HAIGG to GTP\gamma;S—Given that GTP was both necessary and sufficient to drive secretion, we next examined the question of whether [Ca\textsuperscript{2+}], alters the GTP-dependent threshold of the response. Cells were stimulated with HAIGG at room temperature while varying the intracellular concentration of each of the two regulatory components. Initially, the magnitude of the secretory response was determined in the absence of the second regulatory component. The presence of even low levels of GTP\gamma;S in the pipette solution introduced the possibility that some spontaneous secretion could take place independent of either [Ca\textsuperscript{2+}], or HAIGG. To control for this possibility, cells were allowed to equilibrate with GTP\gamma;S for ~10 min, which corresponded to the interval between establishing the whole cell configuration and stimulus addition. HAIGG was added only after the C\textsubscript{m} trace had reached a steady state. Fig. 5 gives a representative time course of an experiment in which the spontaneous GTP\gamma;S (300 \mu M) response is maximal. Note that the response reaches a steady state well within 10 min. All quantitation of the magnitude of secretion was determined as the difference between C\textsubscript{m} values just before the addition of the stimulus and after the response had reached peak values.

A summary of all the experiments investigating the interplay between activated G proteins and [Ca\textsuperscript{2+}], on the amplitude of the secretory response is given in Fig. 6. The stimulus-induced capacitance increase is plotted as a function of [Ca\textsuperscript{2+}], in Fig. 6A. Data were fit with a modified Hill equation, $y = \text{Min} + (\text{Max} - \text{Min})\frac{x^p}{(K_p + x^p)}$, where $p$ is the cooperativity factor or Hill coefficient. Each point represents the average response from 3–6 cells. In the absence of GTP\gamma;S (activation of
Regulation of Macrophage Secretion

Fig. 3. Exocytosis induced by HAIGG is dependent upon G protein activation. The relative time course of changes in membrane capacitance and [Ca\(^{2+}\)] were determined in single cells after stimulation with HAIGG. Experimental conditions were similar to those in Fig. 1 but coupled with simultaneous recording of [Ca\(^{2+}\)] in the presence of [Ca\(^{2+}\)] (fine trace) but failed to induced an increase in [Ca\(^{2+}\)] (bold trace) when [Ca\(^{2+}\)] was increased from 0 (internal solution buffered with 10 mM BAPTA) to 31.5 ± 0.7 pF (n = 3) when the free [Ca\(^{2+}\)] was increased from 0 (internal solution buffered with 10 mM BAPTA) to 600 nM. The response saturated at around 350 nM free [Ca\(^{2+}\)]. Data plotted in Fig. 6B summarize experiments in which the concentration of intracellular GTP\(_S\) was varied in the presence of 10 mM BAPTA to reduce [Ca\(^{2+}\)] levels to near zero in the presence and absence of stimulus addition. The spontaneous response to GTP\(_S\) in the absence of a stimulus ranged from no response at 100 mM to 1.05 ± 0.29 pF (n = 5) at 400 μM. When the cells were stimulated with HAIGG, the amplitude of the secretory response increased from 4.1 ± 0.7 pF (n = 3) to 9.8 ± 0.3 pF (n = 6) when the intracellular GTP\(_S\) concentration was varied from 0 and 300 μM. The response to GTP\(_S\) saturated at 200 μM. We next asked whether the dose-response relationship for [Ca\(^{2+}\)] changes in the presence of GTP\(_S\). In this set of experiments, plotted for comparison with data obtained in the absence of GTP\(_S\) in Fig. 6A, the K\(_S\), indicated as the dotted line, as well as the Hill coefficient remained constant. The observed K\(_S\) in the absence of exogenous GTP\(_S\) was 292 ± 0.4 mM, as compared with 294 ± 2.3 mM in the presence of 100 nM GTP\(_S\) with Hill coefficients of 14.8 and 13.0, respectively. Fig. 6B compares data obtained in the converse case, in which GTP\(_S\) was varied in the presence of 100 nM GTP\(_S\). Under these conditions, the K\(_S\) of the response decreased from 63.4 ± 6.9 μM in the highly Ca\(^{2+}\)-buffered internal solution to 31.5 ± 0.7 μM on a background of 100 nM [Ca\(^{2+}\)]. The Hill coefficients for each condition were approximately equal and were 2.2 and 1.8, respectively. These data are consistent with a model in which increases in [Ca\(^{2+}\)] lower the threshold of a GTP-dependent process. Note that only at the highest GTP\(_S\) concentration (300 μM) would we have underestimated the total response by an average of 0.9 ± 0.1 pF (n = 4) due to spontaneous GTP\(_S\)-induced release in the absence of stimulus.

G proteins by endogenous GTP), the amplitude of the secretory response in response to HAIGG stimulation increased from 0.7 ± 0.2 pF (n = 5) to 6.3 ± 0.4 pF (n = 5) when the free [Ca\(^{2+}\)] was increased from 0 (internal solution buffered with 10 mM BAPTA) to 600 nM. The response saturated at around 350 nM free [Ca\(^{2+}\)]. Data plotted in Fig. 6B summarize experiments in which the concentration of intracellular GTP\(_S\) was varied in the presence of 10 mM BAPTA to reduce [Ca\(^{2+}\)] levels to near zero in the presence and absence of stimulus addition. The spontaneous response to GTP\(_S\) in the absence of a stimulus ranged from no response at 100 mM to 1.05 ± 0.29 pF (n = 5) at 400 μM. When the cells were stimulated with HAIGG, the amplitude of the secretory response increased from 4.1 ± 0.7 pF (n = 3) to 9.8 ± 0.3 pF (n = 6) when the intracellular GTP\(_S\) concentration was varied from 0 and 300 μM. The response to GTP\(_S\) saturated at 200 μM. We next asked whether the dose-response relationship for [Ca\(^{2+}\)] changes in the presence of GTP\(_S\). In this set of experiments, plotted for comparison with data obtained in the absence of GTP\(_S\) in Fig. 6A, the K\(_S\), indicated as the dotted line, as well as the Hill coefficient remained constant. The observed K\(_S\) in the absence of exogenous GTP\(_S\) was 292 ± 0.4 mM, as compared with 294 ± 2.3 mM in the presence of 100 nM GTP\(_S\) with Hill coefficients of 14.8 and 13.0, respectively. Fig. 6B compares data obtained in the converse case, in which GTP\(_S\) was varied in the presence of 100 nM GTP\(_S\). Under these conditions, the K\(_S\) of the response decreased from 63.4 ± 6.9 μM in the highly Ca\(^{2+}\)-buffered internal solution to 31.5 ± 0.7 μM on a background of 100 nM [Ca\(^{2+}\)]. The Hill coefficients for each condition were approximately equal and were 2.2 and 1.8, respectively. These data are consistent with a model in which increases in [Ca\(^{2+}\)] lower the threshold of a GTP-dependent process. Note that only at the highest GTP\(_S\) concentration (300 μM) would we have underestimated the total response by an average of 0.9 ± 0.1 pF (n = 4) due to spontaneous GTP\(_S\)-induced release in the absence of stimulus.

[Ca\(^{2+}\)] and GTP\(_S\) Accelerate the Exocytosis Induced by HAIGG—Given the shift in sensitivity of the secretory response to GTP\(_S\) in the presence of saturating levels [Ca\(^{2+}\)], it was of interest to determine whether the time course of response showed a similar shift. As can be seen in Fig. 7A, the time course of the secretory response as a function of increasing levels of [Ca\(^{2+}\)], was not sensitive to the presence of GTP\(_S\) except for the case in which [Ca\(^{2+}\)] was weakly buffered to 100 nM. C, when [Ca\(^{2+}\)] was strongly buffered, exocytosis induced by HAIGG was inhibited when 1 mM GDP\(_S\) was included in the pipette solution. D, elevated [Ca\(^{2+}\)], did not overcome the inhibition induced by 1 mM GDP\(_S\). E, comparison of the amplitude of the secretory response under experimental conditions, as indicated below each bar. The significance of the difference in the two amplitudes is indicated above each bar as follows. * p < 0.01; # p < 0.05.

DISCUSSION

We have studied quantitatively the regulation of exocytosis in single cells from the murine macrophage cell line J774 using high resolution time-resolved membrane capacitance techniques. We have examined the interaction of cytosolic Ca\(^{2+}\) and activated G proteins in the control of commitment to vesicle fusion in exocytosis after surface receptor binding.

The Isolation of Exocytosis from Phagocytosis—Focal exocytosis and eventual particle uptake are tightly linked and highly regulated sequelae after binding to surface receptors in mononuclear phagocytes. In support of this observation, our studies demonstrate a rapid secretory phase in the capacitance record...
that precedes the phagocytic uptake of the multivalent HAIGG bound to surface Fc receptors for immunoglobulin G. Our capacitance observations are consistent with the studies of Bajno et al. (31), who were able to demonstrate focal exocytosis of endosomal vesicles at sites of phagosome formation. To study the regulation of the exocytic signaling pathway initiated after particle binding to the macrophage surface receptors, we took advantage of the differential temperature and [Ca$^{2+}$]$_i$ sensitivity to separate the two tightly coupled events.

Although it is well established that phagocytosis is highly temperature-dependent (32–34), the question of whether or not phagocytosis is a Ca$^{2+}$-dependent process has been at issue for some time. Fc receptor-mediated particle uptake has been observed to occur in the absence of changes in [Ca$^{2+}$]$_i$ when averaged over the whole cell (35–37). In apparent contrast, other investigations have found that there is a significant reduction in the phagocytic index in cells in which [Ca$^{2+}$]$_i$ is highly buffered (38, 39). A possible resolution to the contradictory findings came from studies suggesting that perhaps two pathways mediating phagocytosis might co-exist in the same cell, a Ca$^{2+}$-dependent mechanism triggered by ligation of Fc receptors and a Ca$^{2+}$-independent mechanism triggered by

FIG. 4. Elevation of [Ca$^{2+}$]$_i$ as well as GTP$\gamma$S is sufficient to induce exocytosis in the absence of an external stimulus. Cells were stimulated either with A23187 (10 $\mu$M) via superfusion of the bath or with 400 $\mu$M GTP$\gamma$S in the pipette solution. Cellular responses were determined in the presence or absence of 1 mM GDP$\beta$S included in the pipette solution as indicated by the symbols over the data from representative cells. Experiments were performed under the same ionic conditions as described in Fig. 3. A, representative recording from a cell stimulated with A23187 in the presence of GDP$\beta$S (1 mM) in the pipette solution. Note that only an increase in [Ca$^{2+}$]$_i$, was observed under these conditions. B, secretory response of a cell stimulated via the inclusion of GTP$\gamma$S in the pipette solution. Note the increase in both [Ca$^{2+}$]$_i$ and Cm. C, representative recording from a cell stimulated via the inclusion of GTP$\gamma$S in the presence of BAPTA (10 mM) in the pipette solution. Note that secretion occurs in the absence of an increase in [Ca$^{2+}$]$_i$. D, comparison of the average capacitance increase under all four conditions, stimulation with A23187 in the presence of Ca$^{2+}$, stimulation with A23187 in the presence of Ca$^{2+}$ and GDP$\beta$S, and stimulation with GTP$\gamma$S in the presence or absence of Ca$^{2+}$. * $p < 0.01$ as compared with the control group.

FIG. 5. Time course of a representative capacitance experiment illustrating HAIGG-induced secretion in the presence of 300 $\mu$M GTP$\gamma$S. Membrane capacitance was monitored for 10 min before adding HAIGG to the bath solution. Establishment of the whole cell configuration is indicated by the arrow. Note the rise in capacitance after $\sim$5 min, presumably due to spontaneous release resulting from the introduction of GTP$\gamma$S into the cell. All quantitation of stimulus-induced changes in membrane capacitance were determined as the differences between the steady state attained after the 10-min equilibration period and the peak release response, as indicated to the left of the $\Delta$Cm trace.

[Ca]$_{out}$ = 0

BAPTA

300 $\mu$M GTP$\gamma$-S

25$^\circ$C

HAIGG

$\Delta$Cm

Cm

200 s

Whole cell break in

4 pF

20 s
activation of C3b/hi receptors (40). Although the possibility of multiple signal transduction pathways controlling phagocytosis might be present in a single cell, it did not resolve questions of regional distribution in [Ca^{2+}] in the stimulated versus unstimulated cell. Subcellular localization studies of [Ca^{2+}], transients subsequently demonstrated that neutrophils ingesting opsonized zymosan particles had the highest level of [Ca^{2+}] in the phagosomes. Calcium in -

Regulation of Exocytosis by [Ca^{2+}], and Activation of G Protein—As with the mast cell (6), our data demonstrate that an increase in [Ca^{2+}], in the absence of G protein activation is not sufficient for secretion after stimulation with HAIGG. In contrast to the mast cell, however, exocytosis in macrophages can result from activating more G proteins or from elevating [Ca^{2+}], at resting levels of G protein activation. Calcium increases the amplitude and decreases the time course of the secretory response in a dose-dependent manner after stimulation with HAIGG. The fact that calcium-dependent increases in the secretory response can be further stimulated in the presence of saturating concentrations of GTP-S suggests that the two signaling elements, activated G proteins and [Ca^{2+}], control secretion in an additive, two-step process. This is further emphasized by the fact that the amplitude and the kinetics of the HAIGG-induced secretory response is dramatically different in the presence and absence of BAPTA (see Fig. 3). Thus, it is to be concluded that both G protein activation and increases in [Ca^{2+}], are essential for an optimal exocytotic response.

A Model for the Exocytosis Regulated by G Protein and [Ca^{2+}], in Macrophage—Our data support a model where the calcium lowers the threshold of a GTP-dependent process in the
control of stimulus-induced secretion in the macrophage (see Fig. 6B). Treatment of the cells with the calcium ionophore A23187 induced marked secretion. GDP-\(\beta\)S blocks this effect, indicating that activated G proteins are necessary for the secretory response. Fig. 8 depicts a two-step model in which a Ca\(^{2+}\)-dependent translocation step enhances the relocation of vesicles from a reserve pool to docked sites at the membrane. The docked vesicles eventually fuse with the plasma membrane in a G protein-dependent step. Activated G proteins appear to be much less effective in moving vesicles from the reserve pool. This model predicts, as was observed in our study, that Ca\(^{2+}\)-dependent transport of vesicles from the reserve pool is rate-limiting in the secretory response. Once the reserve pool has been translocated to a membrane-docked site at saturating \([\text{Ca}^{2+}]_{i}\), the rate of release is rapid and no longer dependent upon further elevation in the GTP\(\gamma\)S concentration. Furthermore, the model predicts that at saturating GTP concentrations the rate of release should be \([\text{Ca}^{2+}]_{i}\)-dependent, reflecting the rate of calcium-dependent vesicle migration from the reserve pool, as was observed in Fig. 6A. And finally, the model predicts that the calcium-dependent step should be upstream of the GTP-dependent step controlling the rate of vesicle supply to the pool of docked granules. Thus, GTP should not alter the \(K_{d}\) of the Ca\(^{2+}\)-dependence, as was observed in Fig. 6A. \([\text{Ca}^{2+}]_{i}\), however, \([\text{Ca}^{2+}]_{i}\) strongly influenced the \(K_{d}\) of the GTP dependence, as was observed in Fig. 6B, presumably augmenting the number of vesicles available for fusion. Further experimental evidence for a GTP-mediated fusion step comes from data in which transient fusion events were observed without external stimulus in BAPTA-buffered internal solutions and high concentrations of GTP\(\beta\)S (see the inset in Fig. 8).

The molecular processes that control docking and fusion of secretory granules in the phagocyte appear to involve interactions between elements of the SNARE complex, which comprise the cellular fusion machinery mediating membrane trafficking and neurotransmitter release in neuroendocrine cells. Isoforms of vesicle-associated membrane proteins, VAMPs, are involved in the recognition of target or t-SNARE proteins associated with the plasma membrane in the docking process. Hackam et al. (44) were the first to describe the presence of VAMP-2 in J774 cells, where it associated with an apparent vesicular compartment. Those molecules, which are recognized by the VAMP proteins on the plasma membrane, are termed t-SNARES. Isoform-specific antibodies have been used to delineate the subcellular distribution of t-SNARE proteins in macrophages. The syntaxins are a subset of the specific t-SNARE-docking proteins thought to control vesicle fusion. Hackam et al. (44) use confocal microscopy to demonstrate that syntaxin 2, 3, and 4 were present not only on the plasma membrane of human and murine macrophages but on intracellular vesicles as well. Although not demonstrated, it is possible that the syntaxins play a role in targeting as well as fusion in the macrophage in the exocytotic release of granular contents into the extracellular compartment. The observation that SNARE proteins also contribute to Ca\(^{2+}\) cooperativity of synaptic transmission (45) suggests that SNARE proteins may be one of the integrative factors by which Ca\(^{2+}\)- and activated G proteins synergistically regulate the exocytosis in the activated macrophage.

Acknowledgments—We thank Dr. H. C. Palfrey and D. A. Hanck for invaluable comments and suggested revisions to the manuscript.

REFERENCES
1. Neher, E., and Marty, A. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6712–6716
2. Fernandez, J. M., Fox, A. P., and Krasne, S. (1984) J. Physiol. (Lond.) 356, 565–568
3. Zimmerberg, J., Curran, M., Cohen, F. S., and Brodwick, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1585–1589
4. Breckenridge, I. J., and Almers, W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1945–1949
5. Breckenridge, I. J., and Almers, W. (1987) Nature 328, 814–817
6. Neher, E. (1988) J. Physiol. (Lond.) 395, 213–214
7. Thomas, P., Suprenant, A., and Almers, W. (1990) Neuron 5, 723–733
8. Horrigan, F. T., and Bookman, R. J. (1994) Neuron 13, 1119–1129
9. Lindau, M., and Fernandez, J. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 84, 1945–1949
10. Parsons, T., Lenzi, D., Almers, W., and Roberts, W. M. (1994) Neuron 33, 875–883
11. Artalejo, C. R., Adams, M. E., and Fox, A. P. (1994) Nature 367, 72–76
12. Hovelevsky, K. O., and Nelson, D. J. (1998) Biophys. J. 75, 2577–2586
13. Nusse, O., and Lindau, M. (1988) J. Cell Biol. 107, 2117–2123
14. Fernandez, J. M., Neher, E., and Gomperts, B. D. (1984) Nature 312, 453–455
15. Pinesifer, J. A., O’Sullivan, A. J., Larbi, K. Y., Tatham, P. E., and Gomperts, B. D. (2000) Biochimie (Paris) 82, 385–393
16. Nusse, O., and Lindau, M. (1990) Biosci. Rep. 10, 93–103
17. Gomperts, B. D. (1996) Annu. Rev. Physiol. 58, 591–606
18. Aridor, M., Rajmillevich, G., Beaven, M. A., and Sagi-Eisenberg, R. (1993)
Regulation of Macrophage Secretion

19. Pinxteren, J. A., O’Sullivan, A. J., Tatham, P. E., and Gomperts, B. D. (1998) EMBO J. 17, 6210–6218
20. Brown, A. M., O’Sullivan, A. J., and Gomperts, B. D. (1998) Mol. Biol. Cell 9, 1053–1063
21. Guo, W., Tamanoi, F., and Novick, P. (2001) Nat. Cell Biol. 3, 353–360
22. Pfeffer, S. R. (1999) Nat. Cell Biol. 1, 17–22
23. Mariot, P., O’Sullivan, A. J., Brown, A. M., and Tatham, P. E. (1996) EMBO J. 15, 6476–6482
24. Hamill, O. P., Marty, A., Neher, E., and Sakmann, B. (1981) Pfluegers Arch. Eur. J. Physiol. 391, 85–100
25. Katnik, C., and Nelson, D. J. (1993) J. Membr. Biol. 134, 213–224
26. Gillis, K. (2000) Pfluegers Arch. Eur. J. Physiol. 439, 655–664
27. Almers, W., and Neher, E. (1985) FEBS Lett. 192, 13–18
28. Fabiato, A., and Fabiato, F. (1979) J. Physiol. Paris 79, 463–505
29. Nelson, D. J., Jacobs, E. R., Tang, J. M., Zeller, J. M., and Bone, R. C. (1985) J. Clin. Invest. 76, 500–507
30. Scepek, S., and Lindau, M. (1993) EMBO J. 12, 1811–1817
31. Bajno, L., Peng, X.-R., Schreiber, A., Moore, H.-P., Trimble, W., and Grinstein, S. (2000) J. Cell Biol. 149, 697–705
32. Heymann, D., Guicheux, J., and Rousselle, A. V. (2001) Histol. Histopathol. 16, 37–44
33. Griffin, F. M., Jr., Griffin, J. A., Leider, J. E., and Silverstein, S. C. (1975) J. Exp. Med. 142, 1263–1282
34. Illinger, D., Poindrud, P., and Kuhry, J. G. (1991) Biol. Cell 73, 131–138
35. McNeil, P., Swanson, J., Wright, S., Silverstein, S., and Taylor, D. (1986) J. Cell Biol. 106, 1586–1592
36. DiVirgilio, F., Meyer, B. C., Greenberg, S., and Silverstein, S. C. (1988) J. Cell Biol. 106, 657–666
37. Greenberg, S., Ksiazek, J. E., Virgilio, F. D., Kaplan, E., and Silverstein, S. (1991) J. Cell Biol. 113, 757–767
38. Young, J.-E., Ko, S., and Cohn, Z. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 5430–5434
39. Hishikawa, T., Cheung, J., Yelamarty, R., and Knutson, D. (1991) J. Cell Biol. 115, 59–66
40. Lew, D. P., Andersson, T., Hed, J., Di Virgilio, F., Puzzau, T., and Stendahl, O. (1985) Nature 315, 509–512
41. Sawyer, D., Sullivan, J., and Mandell, G. (1985) Science 230, 663–666
42. Stendahl, O., Krause, K.-H., Krascher, J., Jerstrom, P., Theler, J.-M., Clark, R. A., Carpenter, J.-L., and Lew, D. P. (1994) Science 265, 1439–1441
43. Hackam, D., Rotstein, O., Sjoalin, C., Schreiber, A., Trimble, W., and Grinstein, S. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 11691–11696
44. Hackam, D., Rotstein, O., Bennett, M., Klip, A., Grinstein, S., and Mandelson, M. (1996) J. Immunol. 156, 4377–4383
45. Stewart, B. A., Mohtashami, M., Trimble, W. S., and Boulianne, G. L. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 13955–13960
Calcium-G Protein Interactions in the Regulation of Macrophage Secretion
Anke Di, Boris Krupa and Deborah J. Nelson

J. Biol. Chem. 2001, 276:37124-37132.
doi: 10.1074/jbc.M105038200 originally published online July 30, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105038200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 45 references, 17 of which can be accessed free at
http://www.jbc.org/content/276/40/37124.full.html#ref-list-1