Ser\textsuperscript{756} of $\beta_2$ integrin controls Rap1 activity during inside-out activation of $\alpha M\beta 2$

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During $\alpha M\beta 2$-mediated phagocytosis, the small GTPase Rap1 activates the $\beta_2$ integrin by binding to a region between residues 732 and 761. Using COS-7 cells transfected with $\alpha M\beta 2$, we show that $\alpha M\beta 2$ activation by the phorbol ester PMA involves Ser\textsuperscript{756} of $\beta_2$. This residue is critical for the local positioning of talin and biochemically interacts with Rap1. Using the CaM (calmodulin) antagonist W7, we found Rap1 recruitment and the inside-out activation of $\alpha M\beta 2$ to be affected. We also report a role for CaMKII (calcium/CaM-dependent kinase II) in the activation of Rap1 during integrin activation. These results demonstrate a distinct physiological role for Ser\textsuperscript{756} of $\beta_2$ integrin, in conjunction with the actions of talin and Rap1, during $\alpha M\beta 2$ activation in macrophages.

Key words: $\alpha M\beta 2$, calcium/calmodulin-dependent kinase II (CaMKII), integrin, phagocytosis, Rap1, talin.

INTRODUCTION

Phagocytosis is the physiological process by which cells internalize particles larger than 0.5 $\mu$m in diameter (e.g. inorganic material, cell debris, micro-organisms, and necrotic and apoptotic cells), and it plays an essential role in development, homeostasis and immune defences [1]. Phagocytic receptors can bind their targets directly or indirectly through opsonins [2] and can exist as constitutively active or inducible receptors prior to binding to their opsonized targets. An example of an inducible phagocytic receptor is integrin $\alpha M\beta 2$, also known as CR3 (complement receptor 3), Mac-1 and CD11b/CD18. This is expressed on the cells of the monocyte/macrophage lineage and polymorphonuclear neutrophils, but also on NK (natural killer) cells, and B- and T-lymphocytes [3]. Phagocytosis through $\alpha M\beta 2$ is a multistep process that sequentially involves receptor-mediated particle recognition via interactions between active integrins and their C3bi ligand, actin-driven uptake and phagosome closure and maturation [4].

Integrins are heterodimeric cell-surface receptors made up of single $\alpha$ and $\beta$ chains. These chains are divided into a large extracellular ligand-binding domain, a single-pass transmembrane domain and a short cytoplasmic tail, and they are bi-directionally regulated. Ligation of the extracellular domain of integrins can trigger ‘outside-in’ signalling pathways that induce changes in cell adhesion, motility and phagocytosis, and promote cell survival and cell-cycle progression [5]. However, the ligand-binding activity of many integrins is regulated by ‘inside-out’ signalling events that affect integrin conformation [6,7]. The cytoskeletal protein talin and the small GTP-binding protein Rap1 are involved in ‘inside-out’ signalling to many integrins [6,7]. In addition, RIAM (Rap1-GTP-interacting adaptor molecule) is reported to be involved in regulation of $\alpha I\beta 3$ activity [8–10].

Previously, we have established that both talin and Rap1, but not RIAM, are essential regulators of ‘inside-out’ activation of integrin $\alpha M\beta 2$ during phagocytosis in macrophages [11,12]. Furthermore, Rap1 activation of $\alpha M\beta 2$ integrin is dependent on binding of talin to the cytoplasmic tail of $\beta_2$ [11,12]. Activation of $\alpha M\beta 2$ by Rap1 was dependent upon residues 732–761 of the $\beta_2$ integrin chain, of which Trp\textsuperscript{747} and Phe\textsuperscript{754} control the binding of the talin head domain to the $\beta_2$ tail [12]. This is essential for optimal inside-out activation of $\beta_2$ integrins in transfected cells [11,13,14]. Also in this region, the triple-threonine residues Thr\textsuperscript{756}–Thr\textsuperscript{760}, which regulate the stable recruitment of active RhoA, actin polymerization and phagocytosis in response to $\alpha M\beta 2$ ligation [15], are dispensable for talin head domain binding and V12Rap1 activation [11].

Fagerholm et al. [16] demonstrated that, in human T-cells, Ser\textsuperscript{756} of $\beta_2$ cytoplasmic tail peptide was phosphorylated after phorbol ester stimulation. This effect was suppressed by the CaM (calmodulin) antagonist W7, suggesting a role for CaM in Ser\textsuperscript{756} phosphorylation. Incidentally, W7 also inhibits the $\beta_1$-mediated adhesion of the monocyte cell line THP-1 to VCAM-1 (vascular cell adhesion molecule-1) and fibronectin in a dose-dependent manner [17]. CaM is a ubiquitous calcium-binding protein that binds and regulates different protein targets and affects different cellular functions. CaM is composed of 148 conserved amino acids, and several common CaM-binding motifs have been identified in CaM-binding proteins, including calcium/CaM-dependent protein kinases [18].

In the present study, we show that Ser\textsuperscript{756} is responsible for the recruitment and activation of the Rap1–talin complex to $\alpha M\beta 2$ integrin. This leads to the activation of the integrin and uptake of C3bi-opsonized RBCs (red blood cells). We suggest that this process is mediated by CaMKII (calcium/CaM-dependent protein kinase II), either by Rap1 activation, Rap1 recruitment to $\alpha M\beta 2$ or by a combination of both processes.
EXPERIMENTAL

Reagents

Sheep RBCs were purchased from TCS Biosciences. PMA, gelatin veronal buffer, Protein G–agarose, guanosine 5′-[γ-thio]triphosphate, guanosine 5′-diphosphate and C5-deficient serum were obtained from Sigma. The CaM antagonist W7 and the CaMKII inhibitor KN62 were obtained from Calbiochem. Rhodamine–phalloidin was obtained from Invitrogen.

The antibodies used in the present study were rat anti-mouse αM (clone 5c6; Serotec); mouse anti-human β2 (clone 6.7; BD-Pharmingen); mouse anti-GFP (green fluorescent protein; clones 7.1 and 13.1; Roche); mouse anti-Myc (clone 9E10; Santa Cruz Biotechnology) and rabbit anti-Myc (clone 71D10; Cell Signaling Technology); rat anti-HA (clone 3F10; Roche); mouse anti-Rap1 (clone 3; BD Transduction Laboratories) and rabbit IgM anti-RBC antibodies (Cedarlane Laboratories). Conjugated secondary antibodies were from Invitrogen, Jackson ImmunoResearch Laboratories (immunofluorescence) or GE Healthcare (Western blotting).

DNA constructs

Eukaryotic expression vectors (pRK5) encoding human wt (wild-type) and mutant αM and β2 have been described previously [11,15,19]. pRK GFP-TFL (GFP–talin) and pRK GFP-TTH (GFP–talin head) were kindly provided by Dr Kazue Matsumoto (Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, U.S.A.) and Dr Neil Bate (Department of Biochemistry, University of Leicester, Leicester, U.K.) respectively.

To generate the β2 S756M and S756D mutants, mutations were introduced into pRK5-β2 by using the QuickChange® site-directed mutagenesis kit (Stratagene), by using the following combinations of primers (mutation underlined): S756D, 5′-GACCGTCGTGGTGGCGTC-3′ and 5′-GCCACCACGACGGTC-3′; S756M, 5′-AACCCCTTTTACAGATGCCACGCCGACGTTAC-3′ and 5′-GACCCTGCTGGTGCGCCATCTTGGAAAGGGTT-3′. Plasmid products were transformed into One Shot TOP10 chemically competent Escherichia coli (Invitrogen) and checked by DNA sequencing (MWG). DNA was later prepared using the Qiagen Endofree maxi-prep kit.

Cell culture and transfection

Cells from murine macrophage J774A.1 and simian kidney fibroblast COS-7 cells (TIB-67 and CRL-1651 respectively; A.T.C.C., Manassas, VA, U.S.A.) were maintained and seeded as described previously [19]. COS-7 cells were transfected using the DEAE-dextran method [19] or by nucleofection (programme A-024; Amaxa Biosystems) and left to express constructs for 48 or 24 h respectively before phagocytic challenge (DEAE-Dextran) or immunoprecipitation (Amaxa).

Phagocytic challenge

IgG- and C3bi-opsonized RBCs [later referred to as IgG- and C3bi-RBCs (C3bi-coated RBCs) respectively] were prepared and used as described previously [15,19], using 0.1 µl (0.5 µl for macrophages) of fresh RBCs per 13 mm glass coverslip. Where needed, cells were pre-treated with inhibitors as follows: W7, 1–100 µM for 15 min, or KN62, 25 µM for 20 min. To elicit ‘inside-out’ signalling, macrophages and αMβ2-expressing COS-7 cells were pre-treated with 150 ng/ml PMA (Sigma) in Hepes-buffered serum-free DMEM (Dulbecco’s modified Eagle’s medium) for 15 min at 37°C as described previously [20]. After challenge with C3bi-RBCs for 30 min at 37°C, cells were washed with PBS to remove unbound RBCs and fixed in ice-cold 4% (w/v) paraformaldehyde for 10 min at 4°C.

Flow cytometry

Transfected COS-7 cells were prepared as described previously [11] and analysed for the relative fluorescence of gated cells, using a FACSCalibur analyser (Becton Dickinson).

Immunofluorescence and scoring

Cells were either stained for surface β2 or permeabilized with 0.2% Triton X-100 and incubated with the appropriate primary and secondary antibodies. β2-expressing cells were distinguished from attached RBCs using different fluorochrome-conjugated secondary antibodies, e.g. a Cy2 (carboxyamine) conjugated donkey anti-mouse antibody for β2 and a Rhodamine Red X-conjugated donkey anti-rabbit antibody to stain opsonized RBCs. Coverslips were finally mounted in Mowiol (Calbiochem), which contained p-phenylene diamine (Sigma) as the anti-fading reagent, and analysed by microscopy using an epifluorescence microscope (BX50; Olympus). Cells co-expressing surface β2 and the different constructs were scored for attached RBCs. The association index is defined as the number of RBCs bound to 100 phagocytes. The measurement of spreading was performed using ImageJ software (National Institutes of Health). The spreading index is defined as the average surface area of 50 macrophages.

The enrichment in GFP- or Myc-tagged proteins at sites of RBC binding was scored by confocal microscopy (LSM510; Zeiss). For these experiments, a minimum of 20 transfected cells per condition were analysed for a discrete local enrichment in marker signal at bound RBCs. Nascent phagocytoses were scored as positive when at least a quarter of the underlying/surrounding area showed significant enrichment, compared with the neighbouring areas.

Significance was tested using Student’s t test. P < 0.05 was considered significant and is indicated by a single asterisk. Insignificant results are indicated by a double asterisk. The lack of an asterisk indicates that no statistical tests were performed for these results.

Immunoprecipitation

Serum-starved transfected COS-7 cells were lysed on ice in a lysis buffer [1% Nonidet P40, 50 mM Tris/HCl, pH 7.6, 150 mM NaCl, 1 mM PMSF and 1 mM DTT (dithiothreitol) plus protease inhibitor cocktail (Roche Applied Science)]. Lysates were incubated for 2 h at 4°C with the appropriate antibodies and Protein G–agarose, followed by three washes in ice-cold lysis buffer, before analysis by SDS/PAGE and Western blotting. Mouse anti-GFP or anti-Myc antibodies (diluted 1:1000) were added for 1 h, followed by HRP (horseradish peroxidase)-conjugated goat anti-mouse antibody. Detection was carried out using the ECL® detection kit (GE Healthcare).

Rap1 pull-down assay

Rap1 pull-downs were conducted as described previously [21], using 20 µg of GST (glutathione transference)–RalGDS–RBD (Rap-binding domain) and 2×10^7 J774A.1 cells per condition. Cells were kept in Hepes-supplemented serum-free DMEM. 

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RESULTS

αMβ2 activation by PMA is regulated by Rap1 and through Ser756 of β2

Previously, we have shown that in both J774A.1 macrophages and COS-7 cells, treatment with the phorbol ester PMA increased binding of C3bi-RBCs via the activity of Rap1, an essential regulator of ‘inside-out’ signalling to integrins [12,20]. We demonstrated that Rap1 acts on the β2 and not the αM chain, through β2 residues 732–761 [12]. In Figure 1(A), αMβ2-expressing COS-7 cells co-transfected with dominant-negative Rap1 (N17) or RapGAP (Rap-specific GTPase-activating protein), which binds and activates Rap1’s intrinsic GTPase activity, turning ‘off’ the signalling cascade, were unresponsive to PMA stimulation (αMβ2 + N17Rap1 with or without PMA, \( P = 0.18 \); αMβ2 + RapGAP with or without PMA, \( P = 0.21 \)). COS-7 cells transfected with wt αM and β2 point mutants W747A or F754A, known to be needed for talin head domain binding and activation [11], were also unresponsive (Figure 1B). Interestingly, expression of the triple-threonine mutant of β2 (to triple-alanine residues), known to recruit active RhoA needed for ‘outside-in’ signalling [15], resulted in increased C3bi-RBC binding, in response to PMA treatment. However, expression of the phosphorylation-insensitive point mutant β2S756M with wt αM, resulted in a lack of C3bi-RBC binding, after PMA stimulation, although surface expression was not affected. These results suggest that Ser756 is required for the regulation of αMβ2, possibly through Rap1 (Figure 1B, and results not shown).

Ser756 of β2 is required for the recruitment of talin

To address the role(s) of Ser756 in the regulation of αMβ2, COS-7 cells were transfected with wt αM and β2 or β2S756M, and Rap1 or talin constructs. These cells were challenged with C3bi-RBCs and the association indices and recruitment of Rap1 or talin were analysed. When wt β2 was replaced with β2S756M, only GFPPTH was able to maintain binding to C3bi-RBCs (Figure 2A). Rap1 was shown previously to act upstream of talin, and they influence each other’s localization to phagocytic cups [12] There is also a correlation between the recruitment of constitutively active Rap1 (V12), full-length talin (GFPFTL) or GFPPTH to αMβ2-dependent C3bi-RBC binding [12]. We found that Ser756 was important for the recruitment of full-length talin. COS-7 cells transfected with wt αM and β2S756M and challenged with C3bi-RBCs showed a significant reduction in recruitment of GFPFTL when compared with cells transfected with wt β2 (Figure 2B, \( P = 0.001 \)). These results suggest that Ser756 is involved in the recruitment of full-length talin, prior to talin head domain exposure and integrin activation.

Rap1 interacts with β2 through Ser756

Since the talin head domain activates αMβ2S756M and Rap1 acts upstream of talin in activating αMβ2, we analysed interactions between Rap1 and β2S756M, using co-immunoprecipitation. We immunoprecipitated GFPPTH, but not GFP from COS-7 cells expressing αMβ2S756M (Figure 3A). To establish whether Rap1 interaction with αMβ2 is mediated by Ser756, we used an anti-αM antibody to immunoprecipitate from COS-7 lysates co-transfected with Myc-tagged wt Rap1, wt αM and phosphorylation-insensitive (S756M) or phospho-mimicking (S756D) mutants of β2. Western blot analysis revealed wt Rap1 co-immunoprecipitated with the phospho-mimicking, but not with the phosphorylation-insensitive point mutant (Figure 3B). We conclude that Rap1 interacts with β2 through Ser756.

PMA- and Rap1-mediated regulation of αMβ2 activity is sensitive to the CaM antagonist W7

Fagerholm et al. [16] demonstrated phosphorylation of β2 Ser756 from human T-cells stimulated with the phorbol ester PDBu (phorbol 12,13-dibutyrate). This effect was suppressed by the CaM antagonist W7 [16]. To explore a role for CaM in αMβ2-mediated C3bi-RBC binding, we used both J774A.1 macrophages and αMβ2-transfected COS-7 cells. J774A.1 cells were pre-treated with W7, stimulated with PMA, challenged with C3bi-RBCs and scored for binding and spreading capabilities. Basal, but not PMA-stimulated binding was significantly decreased at low levels of W7 pre-treatment (25 μM and under, \( P < 0.05 \)) (Figure 4A). However, at 100 μM W7, only PMA-stimulated J774A.1 binding of C3bi-RBCs was significantly decreased (Figure 4A). A similar finding was also observed in the αMβ2-transfected COS-7 cells (results not shown). This effect of W7 is not restricted to binding, as cell spreading was also affected. Unlike binding, spreading decreased in a dose-dependent manner, with basal and PMA-stimulated cells pre-treated with 100 μM W7 showing significant (\( P < 0.05 \)) decrease in spreading...
COS-7 cells were co-transfected with cDNA constructs encoding integrin subunits (wt or with β2S756M) and V12Rap1, GFPTFL or GFPTH as indicated, challenged with C3bi-RBCs, processed for immunofluorescence and scored for (A) RBC association or (B) GFP marker recruitment, as described in the Experimental section. The relative association index shows the relative number of RBCs bound to 100 COS-7 cells (when compared with wt αMβ2, arbitrarily set to 100). The GFP marker recruitment is based on a minimum of 20 transfected COS-7 cells showing local enrichment of marker signal at bound RBCs. Results are expressed as means ± S.D. for at least three independent experiments. *P < 0.05.

(figure 4B). To dissect the effect of W7 on Rap1-mediated inside-out activation, the αMβ2-transfected COS-7 model was used. Increased C3bi-RBC binding was observed when V12Rap1 was overexpressed in αMβ2-transfected COS-7 [12], which was blocked by 100 μM W7 (Figure 4C, αMβ2 with or without V12Rap1, P = 0.14). In αMβ2F754A-transfected COS-7 cells (β2 defective in talin head domain binding), the addition of W7 did not completely abolish binding levels (Figure 4C, P = 0.09). These results suggest the CaM antagonist W7 blocks Rap1-mediated inside-out signalling to αMβ2. A decrease in V12Rap1 localization to sites of C3bi-RBC binding was also seen in W7-pre-treated αMβ2-expressing COS-7 cells (Figure 4D, P = 0.03). We conclude that CaM may be involved in the recruitment of Rap1 to αMβ2 during inside-out signalling.

Rap1 activity is blocked by the CaMKII inhibitor KN62

Previous studies have demonstrated a role for CaMKII during integrin-mediated signalling events [22,23]. We analysed whether CaMKII played a role in Rap1-mediated inside-out signalling to αMβ2. J774A.1 macrophages pre-treated with a CaMKII inhibitor, KN62, displayed a small, but significant, decrease in binding to C3bi-RBCs (Figure 5A, P = 0.002). However, PMA was still able to significantly increase the binding of C3bi-RBCs to KN62-treated J774A.1 macrophages, although not to the same level as the control (Figure 5A, P = 0.02). This suggests that CaMKII functions upstream of the PMA target molecule, presumably Rap1. However, in contrast with cells treated with W7, spreading was not significantly affected (Figure 5B). This suggests a specific role for CaMKII during inside-out activation of αMβ2 in J774A.1 macrophages. To study the effects of KN62 on Rap1 activity, we performed a GST pull-down experiment to detect the levels of GTP-bound Rap1 in macrophages [20]. This was achieved using the Rap1-binding domain of RalGDS, a known Rap1 effector [21]. Glutathione–Sepharose beads coated with GST–RalGDS were mixed with lysates from mouse J774A.1 macrophages loaded with the unhydrolysable GTP analogue, guanosine 5′-[γ-thio]triphosphate or GDP, mimicking active and inactive Rap1 respectively. Western blot analysis (Figure 6A) demonstrated that the GST–RalGDS-coupled glutathione–Sepharose beads were functional, pulling down more GTP-loaded Rap1 compared with the GDP-bound form. To determine the effect(s) of KN62 on Rap1 activation, GST pull-down was repeated, but with KN62-treated J774A.1 macrophages. Figures 6(B) and 6(C) show that lysates of KN62-treated J774A.1 contained less GTP-bound Rap1, compared with the DMSO control. This suggests the CaMKII inhibitor, KN62, inhibits Rap1 activation. We conclude that there is a potential role for CaMKII in the activation of Rap1 during αMβ2 activation.

DISCUSSION

The present study examines the role(s) of Ser756 of β2 (β2S756) in Rap1-mediated inside-out signalling to αMβ2 during phagocytosis. Previously, we showed that the Rap1 activation of αMβ2 was dependent on residues 732–761 of the β2 chain and that Rap1 acts upstream of talin, in a RIAM-independent manner [12]. This β2 region contains two residues known to control talin head domain binding (Trp737 and Phe754), one of which (Phe754) is part of a highly conserved NPXY motif found in cytoplasmic tails of β integrins [24]. These two key residues are essential for maximal inside-out activation of the αMβ2
integrin in macrophages and αMβ2-transfected COS-7 cells [11]. There is also a triple-threonine motif (758–760) that regulates the recruitment of active RhoA to αMβ2 during outside-in signalling, actin polymerization, phagocytosis and recruitment of 14-3-3 proteins [15,25]. We found that αMβ2S756M-expressing COS-7 cells are unresponsive to both PMA stimulation and V12Rap1 activation, but are sensitive to activation by the talin head domain. The mutation of β2S756 to alanine residue had no effect on phorbol ester-induced binding to ICAM-1 (intercellular adhesion molecule 1), a known ligand of the integrin [26]. Our results are also in agreement with research on the homologous Ser756 in β2, which is involved in a variety of integrin-mediated events including adhesion, spreading and migration [27]. The fibroblast cell line GD25 and the teratocarcinoma cell line F9 expressing a β1 mutant containing a dephosphorylated serine residue promoted cell attachment, spreading and migration on laminin [27]. Although Ser756 was essential for Rap1 association with β2, it is only partially involved in talin recruitment to β2. This suggests that other molecule(s) may be involved in talin recruitment, which in turn will lead to Rap1 promoting talin head domain exposure thereby regulating integrin activation.

β2S756 was reported to be phosphorylated after phorbol ester stimulation in T-cells and in reconstituted systems using COS-7 and B-lymphoblastoid cells expressing mutant β2 subunits [16,26]. We found that Rap1 interacts with β2 through the phosphor-mimicking Ser756 residue of the S756D mutant, suggesting the involvement of a kinase. As β2S756 was found not to be phosphorylated by PKC isoforms in T-cells after phorbol ester stimulation [16], other possibilities were considered. The CaM antagonist W7 was known to affect actin reorganization and cell spreading in T-cells challenged with phorbol esters [28]. We found that W7 decreases C3bi-RBC binding, by preventing the recruitment of V12Rap1 to sites of particle binding. In both J774A.1 macrophages and αMβ2-expressing COS-7 cells. Spreading is also decreased, suggesting that W7 blocks a molecule involved in Rap1’s localization to αMβ2 and other integrins. Interestingly, in the monocytic cell line THP-1, W7 inhibits vortex-induced adhesion to VCAM-1 and fibronectin in a dose-dependent manner [17]. Both adhesion mechanisms occur through α4β1 and α5β1 respectively [17]. Recently, it was revealed that CaM forms a complex with ERM (ezrin/radixin/moesin) and that it binds to the cytoplasmic tail of the cell adhesion molecule, L-selectin [29]. With talin also containing a N-terminal FERM (4.1/ezrin/radixin/moesin) domain, and L-selectin clustering involved in inside-out activation of integrins, it is tempting to suggest that these two pathways are linked [29].

A possible candidate for a W7-sensitive serine kinase is CaMKII. CaMKII is a multi-isoform calcium-dependent serine/threonine kinase. CaMKII regulates gene expression, membrane trafficking, secretion, cytoskeletal dynamics, apoptosis and the β3-mediated phagocytosis of apoptotic tumour cells [30]. CaMKII activity is regulated by the binding of calcium-CaM, autophosphorylation of stimulatory and inhibitory sites, and the action of protein phosphatases [31]. Results that implicate CaMKII in various integrin-mediated signalling events are somewhat contradictory, particularly with reference to the use of KN62, a CaMKII inhibitor. Although KN62 specifically inhibited calcium/CaM-dependent protein kinase isofoms [32], and decreased phagocytosis in Lys362 cells [23], it showed no effect on integrin-mediated T-cell–cell adhesion [28]. In addition, KN62 was reported to increase adhesion and spreading in CHO (Chinese-hamster ovary) cells [22]. We found that KN62 blocked C3bi-RBC binding in PMA-stimulated J774A.1 macrophages,
possibly by decreasing Rap1 activity. Interestingly, spreading was unaffected by KN62, suggesting a specific role for CaMKII in αMβ2-mediated particle binding and Rap1 activation.

Although β2 integrin controls a variety of leucocyte functions, including phagocytosis, leucocyte transendothelial migration, motility and the formation of stable immunological synapses, inside-out signalling to β2 is not clearly understood. Other cytoskeletal proteins such as radixin (part of the FERM family of proteins) or vinculin also activate integrins [33,34]. There is also the possibility of an NPXY-independent activation of β2, as inferred by work on the β5 integrin, which regulates the phagocytosis of apoptotic cells [35]. The model we propose is that CaMKII acts on β2 at Ser756, enabling αMβ2 to recruit Rap1, expose the talin head domain and activate αMβ2. CaMKII functions upstream of Rap1, possibly regulating a guanine-nucleotide-exchange factor and thereby promoting the full activation of Rap1. Further work is needed to fully understand the role of CaMKII during αMβ2-mediated phagocytosis.

**AUTHOR CONTRIBUTION**

Emmanuelle Caron and Jenson Lim designed the experiments. Jenson Lim performed the experiments and wrote the manuscript. Neil Hotchin performed the experiments and proofread the manuscript prior to publication.

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