Does neutrophil CD38 have a role in Ca^{++} signaling triggered by β_2 integrin?

To the editor—The recent paper by Partida-Sánchez *et al.*, in which the generation of CD38 deficient mice is reported, shows that CD38 has an important role in the action of neutrophils combating infection[1]. However, we question whether the decreased ability of these knock-out mice to counter infection is the result of a defect in Ca^{++} signaling for neutrophil chemotaxis. A different interpretation of these results suggests that CD38 has a role in the Ca^{++} signaling triggered by a separate aspect of neutrophil behavior.

There are several reasons for suggesting an alternative explanation for increased infection in the CD38-deficient mice. First, although the authors provide evidence for an inhibition of neutrophil chemotaxis in response to formyl-methionyl-leucyl-phenylalanine (fMLP), they found that interleukin-8 (IL-8) signaling remains intact. Therefore IL-8 may have no role in signaling neutrophil chemotaxis in the infected mice. This would seem unlikely as these chemoattractants, together with C5a, are usually involved in driving neutrophil chemotaxis. Also, given that the receptors for these three chemoattractants are of the same class of seven transmembrane-domain, G protein–linked receptors[2], it is possible that they use similar intracellular signaling pathways.

Second, the evidence that Ca^{++} signaling triggered by fMLP is totally dependent on CD38 activity can only be explained by postulating an interplay with IP_3, as there is a wealth of experimental data pointing to IP_3 generation. For example, fMLP generates IP_3 at intracellular concentrations sufficient to release Ca^{++} (ref. 3); blockade of IP_3 receptors inhibits Ca^{++} release triggered by fMLP (ref. 4); and knock-out mice devoid of the IP_3-generating enzymes phospholipase C (PLC) β_2 and β_3 are unable to generate Ca^{++} signals in response to fMLP (ref. 5). In other cell-types, IP_3 interacts with additional second messengers, including the product of CD38 activity, calcium-mobilizing metabolite cyclic ADP-ribose (cADPR), to generate Ca^{++} signals[6].

Third, the evidence that Ca^{++} signaling is crucial for chemotaxis by neutrophils is difficult to reconcile with the fact that fMLP–mediated chemotaxis can be readily dissociated from Ca^{++} signaling. For example, fMLP triggers chemotaxis at sub-nanomolar concentrations, whereas higher concentrations are required for Ca^{++} signaling; chemotaxis in response to fMLP occurs without changes in cytosolic free Ca^{++} concentration[7]; and chemotaxis by neutrophils in response to fMLP is unaffected in PLC $\beta_{2/3}$-deficient mice despite a lack of Ca^{++}-signaling in these mice[5].

Although these authors clearly show that CD38 is important for an aspect of neutrophil physiology and signaling, we suggest the relevant process may be β_2 integrin–mediated responses. The neutrophil β_2 integrin is involved in two key stages in combating infection, both of which have been shown to be signaled through Ca^{++}. β_2 is implicated in neutrophil extravasation, whereupon β_2 integrin engages intracellular adhesion molecule-1 on endothelial cells, and in phagocytosis of C3bi-opsonized bacteria. A defect in β_2-signaling would therefore provide an explanation for both the lack of accumulation of neutrophils in lung and the inability of circulating neutrophils to clear bacteria in *Cd38*$^{-/-}$ mice (the most dramatic difference between wild-type and *Cd38*$^{-/-}$ animals Partida-Sánchez *et al.*[1] found was in circulating bacteria). Furthermore, as the generation of IP_3 in response to β_2 integrin engagement is very low[8], and the characteristics of the Ca^{++} signal are different from those induced by fMLP (ref. 9), an IP_3-independent route to Ca^{++} signaling probably exists. Recently, we have shown that blockade of IP_3 receptors by simple lipid-assisted micro-injection (SLAM)[10] of intracellular heparin fails to inhibit β_2 integrin-mediated phagocytosis and its accompanying Ca^{++} signal, despite preventing Ca^{++} signaling by fMLP (www.uwcm.ac.uk/uwcm/sr/demo3.gif). Experiments of this type point to a non-IP_3-mediated mechanism for the induction of Ca^{++} signaling in neutrophils.

Clearly, identifying the intracellular pathways used by neutrophils in regulating their program of activity is important in understanding neutrophil behavior. Although many roles have been established for cytosolic Ca^{++} in neutrophils, a gap in understanding exists for β_2 integrin signaling by Ca^{++}. The results of Partida-Sánchez *et al.*[1] suggest that a route involving CD38 must now be high on the list of important candidates to be investigated.

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Lund et al. reply—Dewitt *et al.* question our claim that CD38-deficient mice are unable to respond to bacterial infections because *Cd38*$^{-/-}$ neutrophils cannot produce the cADPR and do not mobilize Ca^{++} normally or migrate directionally when stimulated with the bacterial chemoattractant, fMLP (ref. 1). The authors point out that *Cd38*$^{-/-}$ neutrophils can respond to IL-8 (ref. 1) and therefore should be able to migrate to the site of infection. However, mice lacking the N-formyl peptide receptor have the capacity to make and respond to IL-8, yet are highly susceptible to bacterial infections[11]. Thus, formylated peptides released at the site of infection are critical chemoattractants for neutrophils *in vivo*. The authors also state that Ca^{++} mobilization in fMLP-stimulated neutrophils is IP_3 dependent[4] and can be uncoupled from chemotaxis. In addition to IP_3, cADPR and sphingosine-1-phosphate (SPP) mediate intracellular Ca^{++} release in fMLP-stimulated neutrophils[1,12]. We and others[13,14] argue that free cytosolic Ca^{++}, released by IP_3 or SPP, in combination with cADPR induces Ca^{++} release from ryanodine-receptor stores in a process referred to as calcium-induced calcium release. Furthermore, we showed that extracellular Ca^{++} influx in response to fMLP is absolutely dependent on the calcium-induced calcium release response controlled by cADPR and presumably ryanodine receptors[1,15]. Thus, Ca^{++} mobilization in fMLP-stimulated neutrophils is highly complex and involves several different second messengers. Finally, mouse bone-marrow neutrophils that lack either IP_3 or cADPR make poor chemotactic responses to fMLP, thus Ca^{++} release is coupled to chemotaxis for at least this subset of nonelicited and unprimed neutrophils[1,16,17]. Therefore, we believe that our model offers a convincing explanation for our data; however, we do not dis-