COMMENTARY

The behavioural repertoire of neutrophils requires multiple signal transduction pathways

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Signal-response transduction is usually investigated in cells that produce an easily assayed and straightforward response to a well-defined stimulus; the repertoire of the cell is often rather uninteresting. An enjoyable feature of working with neutrophil leucocytes is that their behaviour is far from simple, although at times this can be a mixed blessing. To carry out their primary defensive function *in vivo*, neutrophils must go through a complex sequence of activities, in the correct order, and in the right places. In an acute inflammatory response neutrophils adhere to, and move over, the luminal surface of endothelial cells, penetrate the endothelium, and move through connective tissue towards the site of damage. Having arrived, and stopped moving, neutrophils then engage in phagocytosis or secrete hydrolytic enzymes to digest large foreign objects (Lackie, 1982). The early stages require changes in adhesive and motor capacity, the late stages of the process are marked by a dramatic activation of the metabolism of the cell. Activities that are essential in the late stages would be inappropriate, and destructive of normal tissue, in the early stages.

Are the different activities induced by quantitative changes in the signal, or is there a qualitative change, a different signal being responsible for triggering the late events? Alternatively, might it be that quantitative changes in the signal lead to an altered balance between two different signal-response transduction systems?

Once we give our attention to a cell that has a complex behavioural repertoire, then straightforward schemes for signalling seem less satisfactory; a system of control based upon quantitative changes in the concentration of a single second messenger in the cytoplasm would have to be very finely tuned. All-or-none events, like bursts of secretory activity, are relatively easy to control; getting a cell from the circulation to a site of tissue damage requires more sophistication. To use a favourite analogy: getting a car started is not usually difficult, but driving it to work through the rush hour is more demanding. Not only is the task faced by the neutrophil complex, but the stimulus is unpredictable in character. A defence system must be designed to handle diverse, or even novel, situations, and a variety of different signals must elicit a standard set of responses. Thus interesting cells like neutrophils will require much more complex control systems than, for example, neurones, which stay in one place and make predictable responses to familiar signals. Neglect of the subtleties of real cell behaviour allows unified and all-embracing, but unrealistic, hypotheses of signal-response coupling to gain a hold on our collective imaginations. In an attempt to redress the balance, I will try to show here the evidence that multiple signal-response systems must be operating in neutrophils.

In the case of the so-called chemotactic peptides (formylated tripeptides like formyl-methionyl-leucyl-phenylalanine, fMLP), we know that low levels will induce a transient rise in adhesion (O'Flaherty *et al.* 1978), elicit shape changes (Shields & Haston, 1985), alter the G/F-actin equilibrium (Howard & Oresajo, 1985; Sha'afi *et al.* 1986), and stimulate movement (Zigmond *et al.* 1981). Concentrations at least an order of magnitude higher are needed to stimulate the late events of metabolic activation and the induction of release of granule contents. The early events, such as movement and orientation, are mediated through a specific receptor, which has a K_d of around 2×10^{-8} M, and the chemokinetic effect of fMLP is greatest at about 5×10^{-9} M. The late events require concentrations of 10^{-7} to 10^{-6} M, and it seems improbable that they can be controlled through the same system. Furthermore, some early events are transient, for example the increases in adhesion and in membrane-associated F-actin, which are over within 5–10 min at most, whereas other activities such as movement, and possibly gradient perception, are sustained for hours.

In order to investigate the signalling system or systems it is essential to have assays for all the activities of neutrophils, not just the late events, which are more

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commonly tested. These assays are probably unfamiliar to the biochemist, involving as they do the analysis of the adhesive and locomotory behaviour of whole cells. Cell behaviourists will, however, recognize that the flow chamber adhesion assay (Forrester & Lackie, 1984), the automated cell tracking system for neutrophils (Dow *et al.* 1987), and the collagen gel assay (Brown, 1982) serve to model the early activities of neutrophils in an inflammatory response and could be used for this purpose. Coupled with a chemiluminescence assay for metabolic activation (Bender *et al.* 1983), both the early and late events in inflammation can be investigated and quantified.

The late events induced by high concentrations of fMLP are probably signalled through phosphatidyl inositol turnover and the activation of protein kinase C, the scheme so convincingly advocated by Berridge (1987, for review) and Nishizuka (1984). Not only can phorbol myristate acetate, which is thought to activate protein kinase C directly, produce similar responses, but metabolic activation and superoxide release induced by fMLP can be inhibited by pertussis toxin (Becker *et al.* 1985; our own unpublished work), implying that there is a G-protein link in the transduction pathway. The fMLP response is enhanced by the addition of a diacyl-glycerol kinase inhibitor (R59022), which would allow the accumulation of diacyl-glycerol and thereby stimulate protein kinase C, although R59022 is ineffective on its own (unpublished results). What does come as a surprise is that fMLP-induced and phorbol myristate acetate-induced chemiluminescence show the opposite sensitivity to the addition of exogenous phospholipase A2 (PLA2), the fMLP response being potentiated, the phorbol myristate acetate-induced chemiluminescence being inhibited markedly (Lackie & Lawrence, 1987). This is hardly what would be expected of a simple phosphatidyl inositol/protein kinase C system. If phorbol myristate acetate acts on the protein kinase C system, which is normally activated by phosphatidyl inositol turnover, then does fMLP act exclusively through this pathway? Perhaps fMLP also acts through an endogenous PLA2 system, as suggested by Bormann *et al.* (1984), a possibility that is reinforced by the observation that lipocortin, a plasma inhibitor of PLA2, is synthesized in increased amounts when leucocytes are stimulated by anti-inflammatory steroids (Blackwell *et al.* 1980), and is inactivated by autoantibody in some chronic inflammatory diseases (Hirata *et al.* 1981).

Although PLA2 differs from phospholipase C in being much less substrate-specific, both phospholipases produce two potential second-messenger molecules, and there are now suggestions that PLA2, like protein kinase C, may be controlled by G-proteins (Burgoyne *et al.* 1987). Phosphatidyl inositol-specific phospholipase C will generate diacyl-glycerol and

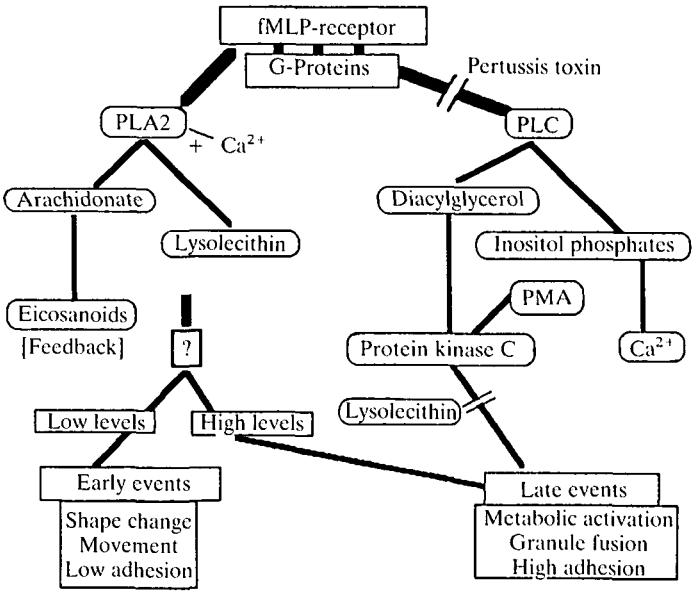

Fig. 1. A schematic representation of the possible and probable links between the various signal-response pathways discussed. Where lines contact boxes the link is more definite. Since the connection between the PLA2 system and activation is unclear, a question mark has been inserted into the scheme. Not all interactions are shown.

inositol trisphosphate; PLA2 will generate a variety of fatty acids and lysophospholipids. If the fatty acid is arachidonic acid then it might provide a starting substrate for the production of eicosanoids, many of which are inflammatory mediators and could produce a positive feedback in the system; the lysophosphatide, as a potential fusogen, could have effects on membrane dynamics. The addition of exogenous arachidonic acid will enhance both fMLP- and phorbol myristate acetate-induced chemiluminescence, whereas lysophosphatide has no effect on the fMLP-induced response, but inhibits the response to phorbol myristate acetate. The two agonists show different responses both to PLA2 and to one of its products. Fig. 1 is an attempt to summarize this in diagrammatic form. Another difference between the agonists is that phorbol myristate acetate will induce only the release of specific granules (Bainton, 1973), whereas fMLP will cause both azurophil and specific granules to be released.

The hypothesis of multiple signal-response pathways becomes more convincing when the effects of these two agonists on the early events of inflammation are considered. The most convenient activity to discuss, and the one for which we have most information, is the movement of neutrophils over a two-dimensional substratum. Several studies have now shown that fMLP increases the speed of neutrophils, both over a substratum and in a three-dimensional matrix, and speed is further increased by the addition of PLA2, which on its own has no effect. The diacyl-glycerol kinase inhibitor

R59022 does not enhance movement, rather the reverse, nor does pertussis toxin seem to inhibit (unpublished results). In contrast, phorbol myristate acetate will almost completely suppress movement over a substratum, even at doses substantially lower than those required to induce chemiluminescence. The inhibition of locomotion by phorbol myristate acetate does not seem to be relieved by PLA2, and is not simply a consequence of an increase in adhesion, because the shape change of neutrophils in suspension is also altered, though not suppressed (Roos *et al.* 1987).

The effects of fMLP on the early and late responses are consistent with a model that requires only quantitative change in the signal, though the high-concentration effects seem to be much more sensitive to pertussis toxin, implying that a protein kinase C system is coming into operation as well as a PLA2-mediated system. Our current working hypothesis is that the early events are triggered through a system that involves an endogenous PLA2, and that the late events also involve a protein kinase C system. Activating the protein kinase C system alone induces some, but not all, of the late events, and will override the early activities. Perhaps the inhibition of protein kinase C by the products of PLA2 activity may prevent the early systems being hindered by the phorbol myristate acetate-type effects, which are appropriate at a later stage.

The work discussed above is still in progress, and many questions remain, but it is introduced to make a more general point. It is my belief that only by looking at cells with a complex behavioural repertoire will the full intricacies of signalling systems be revealed, and only by using more complex cell behavioural assays will the more subtle aspects of control be recognized. Had we concentrated on the dramatic and easily assayed end events, the release of lysosomal enzymes and the production of active oxygen species, then we would have seen only a small part of the picture. Neutrophil behaviour is interesting in its own right and is also of some clinical importance; blocking the early events in inflammation might be a better therapeutic strategy than trying to inhibit the later stages.

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