COMMENTARY

Gradient perception by neutrophil leucocytes, continued

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The mechanism by which neutrophil leucocytes exhibit chemotaxis continues to be the subject of debate. In recent articles in this journal (Vicker *et al.* 1986; Haston & Wilkinson, 1987) the question of whether neutrophils can perceive spatial attractant concentration gradients has been discussed. The purpose of this brief note is to give attention to some information that should help clarify some of the points raised by these articles.

Vicker *et al.* (1986) reported that, in order for neutrophils to exhibit directional orientation bias, they need to encounter a chemoattractant concentration that increases with respect to time, and may not be able to detect a stable spatial concentration gradient. This conclusion was based on comparison of cell movement in gradients possessing a temporally increasing component to movement in gradients lacking a significant temporally increasing component. It is not difficult to show, however, that their results could be explained equally well by the spatial gradients present in these experiments. Calculations based on analysis of molecular diffusion processes (e.g. see Lauffenburger & Zigmond, 1981) showed that the magnitudes of the spatial gradients present in the experiments with a temporally increasing concentration were great enough to stimulate an orientation response (cf. Zigmond, 1977), while the magnitudes of the spatial gradients present in the experiments lacking a temporal increase in concentration were too small to stimulate orientation. That is, roughly 1 % concentration change over about 10 μm distance is needed to induce a significant level of orientation at optimal peptide chemoattractant concentrations. In the filter experiments performed by Vicker *et al.*, however, we calculate that the gradient present after the 10 min incubation at 0 °C is only about 0·6 % over 10 μm and decreases to less than 0·3 % at 60 min. Similarly, in the experiments under agarose that they present, we calculate that there is less than a 0·1 % gradient present over 10 μm after the 2-h incubation at 0 °C. In contrast, the spatial gradients present during the initial incubation periods were calculated to be greater than 1 % over 10 μm in both experiments. *Thus, the effect that Vicker* et al. *attribute to temporal gradients can be explained equally well by the spatial gradients present.* Hence, we believe that their results do not require the conclusion that neutrophils need temporally increasing attractant concentrations in order to orient.

On the other hand, we are aware of experimental evidence suggesting that neutrophils do *not* need to encounter temporally increasing chemoattractant gradients in order to exhibit directional movement. First, recent experiments (Lauffenburger *et al.* 1987) using an under-agarose population-migration assay with uniform levels of fNLLP demonstrated enhanced cell migration as the cell density in the well was increased. A likely explanation is that peptide uptake at the high cell densities created significant spatial gradients of the attractant, to which the cells responded chemotactically. Quantitative calculations of the cell density needed to create a large enough spatial gradient by attractant uptake (Lauffenburger *et al.* 1987), using previous measurements of the cell uptake rate constant (Zigmond *et al.* 1982), are in agreement with the experimental observations. In this case, the attractant concentration would be *decreasing with time*, yet the cells show a dramatic response. Second, we have performed experiments using the Zigmond chamber comparing orientation behaviour of cells that experienced spatial gradients established by increasing the attractant concentration in the source well, to behaviour of cells that experienced gradients formed by decreasing the attractant concentration in the sink well. In both experiments similar spatial gradients were seen by the cells; however, the attractant concentration was *increasing with time* in the first experiment but *decreasing with time* in the second experiment. Orientation levels achieved in the two experiments were comparable and at typical values. Thus, the direction of the macroscopic temporal change in attractant concentration does not appear to matter to the cells.

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This is not to say that neutrophils might not perceive spatial concentration gradients by a temporally mediated, 'pseudo-spatial' mechanism. One possibility is the local protrusion of a pseudopod, with protrusion up a spatial gradient translated into a temporal increase in attractant binding by receptors on that pseudopod (Gerisch *et al.* 1974; Zigmond, 1982). The key point we are offering here is that the attractant concentration itself need not be increasing with respect to time.

We do believe, along with Haston & Wilkinson (1987), that there is an important probabilistic aspect to the perception of spatial gradients (although our view of the underlying mechanism is very different), since receptor–attractant binding as well as further signal transduction and response events are surely stochastic in nature. This belief is, in fact, supported by previous theoretical calculations demonstrating that experimentally observed orientation behaviour can be accounted for by consideration of kinetic 'noise' in the gradient signal perception process (Tranquillo & Lauffenburger, 1986, 1987).

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