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Leukocyte movement in the presence and absence of chemoattractant gradients and its implications for clinical studies

PC Wilkinson

Department of Bacteriology and Immunology University of Glasgow Western Infirmary Glasgow, G11 6NT, U. K.

During the past decade or so, the measurement of leukocyte chemotaxis has become a routine investigation in many clinical immunology laboratories, and there is now a large literature documenting abnormalities in various diseases. Despite this, there is little substantial evidence that such abnormalities are of much importance in the pathogenesis of disease, and understanding of the pathology of leukocyte locomotion is confused. A possible exception may be made in the case of patients with defects of adhesive proteins such as LFA-1, CR3 and P150:95, but these are really defects of adhesion, not of locomotion. Obviously if cells in the circulation cannot adhere to endothelium, they cannot migrate across it. What evidence there is suggests that, in circumstances where adhesion is not required for locomotion, these cells can move normally [1, 14]. Lymphocytes normally locomote without adhering [7].

One of the difficulties with the clinical measurement of chemotaxis is methodological. The two simplest and most popular assays are the micropore filter assay and the agarose assay. These assays are useful for measuring how whole cell populations respond to a stimulus but they tell us little about the locomotor behaviour of individual cells, or the details of how attractants modify that behaviour. It is therefore worthwhile to outline this behaviour and I shall devote the remainder of this article to that task.

How do leukocytes detect gradients

Two reactions which cells show in response to chemical stimuli are generally accepted and have been defined [12]. Chemotaxis is a reaction which determines the direction of locomotion of cells and usually the cells migrate directionally toward the source of a chemical gradient. Chemokinesis is a reaction which determines the speed or the frequency of turning of cells. For practical purposes when dealing with leukocytes, the major form of chemokinesis to be considered is that which determines the speed of locomotion of cells (orthokinesis), and

I shall not discuss other forms of chemokinesis.

We should ask how molecules in the environment of the cell determine the direction and the speed of cell movement and whether these two parameters are determined by the same or different mechanisms. An even more basic question (which will help to answer the first two questions) is whether cells move *only* in response to signals from external molecules or whether they have some internal machinery which allows them to move around at random in the absence of external stimuli. Time-lapse films of leukocytes circulating freely in the blood without impedance and without adhering suggest that these cells are spherical in shape. Moreover, leukocytes of all types, when prepared carefully *in vitro* from blood, and maintained in suspension without being allowed to adhere to a surface, are also spherical. This spherical morphology is typical of an immotile cell in suspension. If, to spherical neutrophils in suspension, an attractant is added at uniform and optimal concentration (e.g. f-Met-Leu-Phe or C5a at 10^{-8}M), almost the entire population will undergo a change in shape during the next few minutes [16]. The cells take up a polarized morphology with a ruffled leading edge (lamellipodium) and a body tapering to a narrow tail. This is typical locomotor morphology. If these cells, in suspension, are observed by timelapse cinematography, contractions, visible as constriction rings, are seen to pass anteroposteriorly down the cell and if the cells are allowed to move over a surface, the cell moves forward with the constriction as a fixed point relative to the substratum [15, 8]. Moreover, a remarkable redistribution of membrane proteins can be seen as the cells change shape. Many membrane proteins become redistributed to the front of the cell. These include Fc receptors [18, 20], C3b receptors [16], Thy-1 on mouse lymphocytes [16] and f-Met--Leu-Phe receptors [17]. This distribution is ligand-independent and is probably a redistribution of non-cross-linked proteins (and thus distinct from capping). At the same time, polymerized actin is seen to form rapidly in the cytoplasm at the leading edge of the cell [6].

It is remarkable that the shape--change seen in uniform concentrations of attractant is identical to the shape--change seen in gradients of attractant. The only difference is that in uniform concentrations, the long axes of the different polarized cells in the population are randomly oriented, whereas in gradients, the long axes of the different cells tend to be oriented perpendicular to the gradient source. What we should then ask is how cells are able to polarize at all in a uniform attractant concentration at which receptors should be able to make rapid contact with many molecules of ligand, and how the cells orient in a gradient.

The description that I have given of leukocyte behaviour in the presence and absence of a ligand suggests that leukocytes do not show any internally signalled locomotor behaviour, but

only exhibit polarization and motility when stimulated from outside. This stimulus takes the form of a ligand binding to a cell-membrane receptor and passing a signal to the interior locomotor machinery of the cell. We have argued that the easiest explanation of cell polarization in a uniform concentration of attractant is a 'first--hit' mechanism [9, 19]. The cell responds by polarizing with its front end at the point where the first signalled event (ligand-receptor binding followed by transduction and formation of a second messenger) takes place. Once this signal has been received, the cell changes from a spherical to a polarized shape and, simultaneously, the receptors redistribute to the forward pole of the cell. The cell will locomote in the direction of the first stimulus and this locomotion will show'persistence' i.e. the cell will follow the same path for some time (since the receptors are at the forward end and subsequent hits are therefore likely to be at that end). If this is the case, it would be expected that cells exposed to high (supraoptimal) concentrations of attractant would be 'hit' simultaneously at many points and would have difficulty establishing a polarity. This is exactly what happens, e.g. if neutrophils are exposed to 10^{-6} M f-Met-Leu-Phe, they show several pseudopods, poor polarity, and poor persistence of locomotion [16].

This suggests that the direction in which cells become polarized is essentially determined by chance. Cells in a uniform and optimal concentration of attractant stand an equal chance of being stimulated at any point on the cell surface and the direction of their polarization and therefore of their subsequent locomotion is random. In a gradient however, the number of attractant molecules is higher on one side of the cell population than on the other, and the chance of 'hits' is therefore higher on one side. Thus chance determines that most of the cells will polarize towards the gradient source. It follows that there is no need for different mechanisms for stimulation of chemotactic and chemokinetic responses. The basic action of chemotactic factors is to stimulate cell polarization and locomotion. Whether the cells show chemotaxis or chemokinesis is secondary and is determined by the disposition and concentration of attractant molecules in the environment, and not by specialized properties of the cell.

Clinical abnormalities

If this is the case, we can start a rational examination of possible levels at which clinical abnormalities of locomotion might exist. We would expect these abnormalities to exist at some point in the pathway between signal reception and the locomotor response, e.g. receptors, transduction molecules, effector molecules (kinases, phospholipases), second messenger generation, cytoskeletal assembly. We would not expect to find defects of chemotaxis isolated from defects of chemokinesis, since the mechanisms for both are the same, although conside-

rable ingenuity and trouble have gone into the search for such defects and there are many reports of them. The difficulty here, once again, is methodological. The filter assay and the agarose assay are not ideal for distinguishing chemotaxis from chemokinesis because they do not allow a direct measure of the direction and speed of cells. This is best achieved by visual analysis of the paths of individual cells and there is considerable literature about these analyses (see for example, 5, 10). There are some pointers to future work in the clinical literature. For example, there is a substantial defect of locomotion of the lymphocytes from patients with chronic lymphocytic leukaemia [11, 20] and these cells show a defect of membrane receptor redistribution which may be related to the fact that they do not polarize properly [4]. It may be that this is a property of immature cells under normal conditions as well. Neutrophil and monocyte precursors, which are conveniently studied using cell lines such as HL-60 and U937, are non-motile, but when they are induced to mature, they acquire locomotor capacity. During maturation they also acquire membrane receptors for chemotactic factors such as f-Met-Leu-Phe or C5a [2, 13]. Finally the possibility that cytoskeletal abnormalities lead to chemotactic defects is pinpointed by the studies of a patient whose leukocytes lacked an actin-binding protein and were unable to polymerize actin [3]. Unfortunately, such patients are rarely available for study.

It is unfortunate that the term 'chemotactic factor' has become so current among workers in this field. It is quite clear that these factors are not just chemotactic factors but have multiple effects on locomotion, metabolic activation, etc. I have tried to argue here that their basic effect is to stimulate leukocyte locomotion. Chemotaxis and chemokinesis are merely forms of locomotor response which are determined by the environment that the cell finds itself in, i.e. a varying absolute concentration or a varying concentration gradient of attractant.

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