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Mastering time and space: immune cell polarization and chemotaxis

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Abstract

Many immune cells can detect the direction and intensity of an extracellular chemical gradient, and migrate toward the source of stimulus. This process, called chemotaxis, is essential for immune system function and homeostasis, and its deregulation is associated with serious diseases. Chemotaxis is initiated by chemoattractant binding to heterotrimeric G protein-coupled receptors, which translate the gradients into accurate directional migration. A necessary step in this process is cell polarization, the acquisition of functional and spatial asymmetry. The use of new imaging technologies enables analysis of spatial and temporal changes in the activity of proteins and membrane domains involved in polarization and chemotaxis. We discuss the sometimes contradictory evidence available and the emerging molecular model for immune cell polarity and chemotaxis.

Keywords: Dendritic cells; Chemoattractant molecule; Actin polymerization

1. Introduction

Cell migration is a crucial process in many important biological events, including embryo implantation, developmental patterning, axon guidance and wound healing. In the immune system, migration is a prominent component of the leukocyte-mediated removal of invading microorganisms and infected cells. Chemokines and their receptors regulate leukocyte migration in response to inflammation, as well as the homing of dendritic cells and naïve lymphocytes to secondary lymphoid organs, which enables adaptive immune responses and tissue homeostasis. As a corollary to the role of cell migration in establishing a functional immune system, deregulated movement of immune cells is associated with the development of many pathological conditions, including autoimmunity, chronic inflammation, immunodeficiency and cancer.

Immune cells can migrate in three ways. Chemotaxis refers to a cell's ability to move toward a source of a soluble chemoattractant molecule; chemokinesis is the increase in random, undirected cell motility, and haptotaxis is cell migration toward a gradient of an immobilized substrate. Whether these processes are governed by the same molecular mechanisms is not known, but three basic principles can be applied to all of them [1]. First, cells must become polarized to migrate. This means that migrating cells are morphologically and functionally asymmetric, with two opposite compartments: the leading edge at the front and the uropod at the rear. Second, migration is a cyclic process, involving the extension of protrusions (pseudopodia, lamellipodia and filopodia) at the cell front and retraction at the cell back. Finally, the ability to move is balanced by cell adhesion to the extracellular matrix (ECM), at least in vitro. On overly sticky

Abbreviations: Arp2/3, actin-related protein; CRAC, cytosolic regulator for adenylyl cyclase; DOCK-2, downstream of Crk-180 homolog-2 protein; ECM, extracellular matrix; ERM, ezrin-radixin-moesin; F-actin, filamentous actin; fMLP, formyl-methionyl-leucyl-phenylalanine; FRET, fluorescence resonance energy transfer; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; GM1, ganglioside-monosialic acid; GPCR, G protein-coupled receptor; IQGAP, IQ motif containing GTPase activating protein; LFA-1, lymphocyte function-associated antigen; L-raft, leading edge raft; MLC, myosin light chain; MT, microtubule; MTOC, microtubule organizing center; PAK1, p21-activated kinase; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; Ptdlns, phosphatidylinositol; PTEN, phosphatase and tensin homolog on chromosome 10; RAPL, regulator for adhesion and polarization enriched in lymphoid tissues; ROCK, Rho-associated coiled-coil kinase; U-raft, uropod raft; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP verprolin-homologous protein

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surfaces, cells flatten but cannot crawl, whereas on insufficiently sticky surfaces, cells cannot generate traction forces to move forward.

Our present understanding of cell migration derives from studies of different cell types, modes of migration, and environments. This variation explains the discrepancies among studies and complicates full comprehension of the process. Some components are nonetheless shared under different conditions, indicating solid elements in the cell migration program. In this review, we will focus on the spatial and functional polarization of immune cells engaged in chemotaxis. It is inevitable, but also helpful, to look at other cell types that polarize in response to attractants, as this highlights the basic molecular mechanisms involved in cell polarity.

2. Basic principles of chemotaxis

During chemotaxis, a cell must determine the general direction of the signal source and orient itself accordingly. This is possible since chemotaxing cells are extremely sensitive to small differences in chemoattractant concentrations between the cell front and rear; for example, neutrophils can identify a 2% difference in chemoattractant concentration over a 10 μm distance (the length of the cell) [2]. Shallow extracellular chemoattractant gradients are amplified into steep intracellular gradients (a process termed directional sensing), leading to spatial and functional asymmetry. Based on currently available techniques for chemotaxis analysis (Fig. 1), two major models have been proposed for directional sensing [3]. The spatial sensing model involves the detection of differences in chemoattractant concentrations across the cell, whereas temporal sensing implies that cells sense time-dependent changes in concentration as they move. Small motile cells such as bacteria rely mainly on temporal sensing, but eukaryotic cells use spatial and temporal sensing simultaneously. For both mechanisms, receptor modification and downregulation are key adaptations to prevent receptor saturation.

Directional sensing is regulated by the interplay of various signaling pathways and other cellular events, presumably connected to the actin polymerization machinery, which is the basal mechanism of pseudopod formation [4]. In the absence of chemoattractants, a cell extends pseudopodia in more or less random directions. In the presence of a chemotactic gradient, however, actin polymerization is biased to produce more pseudopodia at the cell front, defined as the site of the highest chemoattractant concentration. In neutrophils and lymphocytes, this polarity is very persistent even in a homogeneous chemoattractant solution; a 180° change in gradient direction usually leads cells to make U-turns [2]. This contrasts with *Dictyostelium* cells, in which polarity is a fairly transient state, and a cell usually develops a new leading edge when the gradient source is changed. In the following sections, we will scrutinize the molecular machinery that underlies persistent cell polarization and migration in chemoattractant gradients.

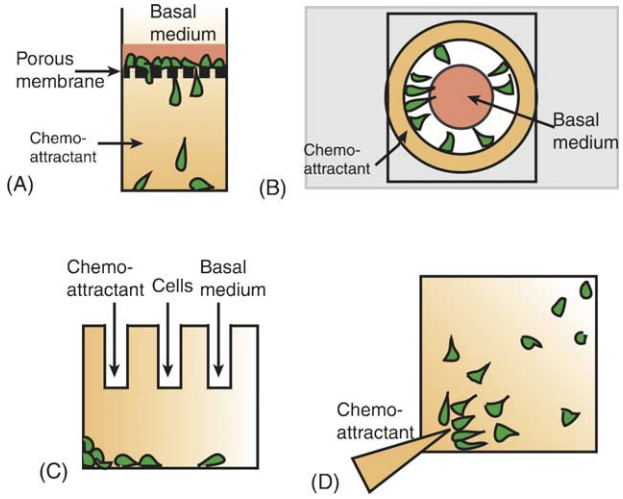

Fig. 1. Methods for studying chemotaxis. (A) Boyden chamber assay. Cells are plated on a porous membrane in a well, which is placed over a chamber containing the chemoattractant; a gradient is formed by passive diffusion. The magnitude of the chemotactic response is quantified by comparing the number of cells that traverse the filter to the basal levels of transmigration in the absence of stimulus. Transwell assays are appropriate for screening and easy to perform, but can be misleading, since results are influenced by changes in adhesion, motility, or spatial orientation. Moreover, they do not permit measurement of cell migration speed or small changes in the direction of moving cells. (B) The Dunn direct-viewing chemotaxis chamber is built on the principles of the Zigmond chamber, and offers the possibility of imaging migration within a linear chemoattractant gradient. (C) The under-agarose cell migration assay for chemotaxis and chemokinesis creates an environment with complex spatial and temporal chemotactic gradients; it can be adapted for real-time imaging and fluorescence studies. (D) Real-time imaging experiments create a diffusion gradient using a point source of chemokine, such as a pipette tip. This method was used to show chemotaxis in *Dictyostelium*, neuronal growth cones, and neutrophils. Coupled with GFP technology, such assays permit real-time analysis of membrane receptors and signaling intermediates in moving cells.

3. Chemoattractant receptors: sensing devices

Chemoattractants are the spatial signals that initiate and maintain cell polarization during chemotaxis. Most known chemoattractants bind to seven-transmembrane receptors coupled to heterotrimeric G proteins (GPCR) that contain the Gα; subunit and mediate chemotaxis through Gβγ subunit release. Data suggest that GPCR also govern cell polarity and chemotaxis in response to different non-GPCR agonists [5]. This highlights the importance of dissecting GPCR signaling to understand the mechanisms of cell polarity.

There is debate as to the location of GPCR in polarized immune cells. It was initially reported that formyl-methionyl-leucyl-phenylalanine (fMLP) receptors in neutrophils, and the CCR2, CCR5, and CXCR4 chemokine receptors in some lymphocyte subsets were located preferentially at the leading edge of polarized cells (for review, see [6]). Nonetheless, experiments using GFP-tagged receptors suggested that C5a receptors on human neutrophils did not concentrate at the leading edge of polarized moving cells [7]; although receptor number was apparently enriched in some polarized cells, this