Review

Amoeboid movement in protozoan pathogens

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ABSTRACT

Entamoeba histolytica, the causative agent of amoebiasis, is a protozoan parasite characterised by its amoeboid motility, which is essential to its survival and invasion of the human host. Elucidating the molecular mechanisms leading to invasion of human tissues by *E. histolytica* requires a quantitative understanding of how its cytoskeleton deforms and tailors its mode of migration to the local microenvironment. Here we review the wide range of methods available to extract biophysical information from amoeboid cells, from interventional techniques to computational modelling approaches, and discuss how recent developments in bioimaging and bioimage informatics can complement our understanding of cellular morphodynamics at the intracellular level.

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1. Background: mesenchymal *vs.* amoeboid motility

The chemical nature of the extracellular matrix (ECM) and the architectural complexity of the tissues are important parameters to evaluate and conclude on the mechanisms that govern the spatial displacement of cells. These parameters are not uniform; even more, they may vary rapidly as a function of the topography and functions of the tissues in which cells move [1,2]. Therefore, motile cells must respond promptly to the dynamics of the environment to optimise its displacements. In 3D environments, two modes of migration are largely recognised: mesenchymal migration, characterised by proteolysis of the ECM, and amoeboid migration, where cell deformation allows the cells to cross the ECM in the absence of proteolysis [3–5].

Mesenchymal cells form highly structured, adhesive complexes through specialised surface receptors (*e.g.* integrins) and the ECM [3]. Their cytoskeleton provides the molecular mechanism sustaining the formation and function of these adhesive complexes as well as those formed with neighbouring cells. The stability of adhesive complexes implicitly determines the morphology of mesenchymal

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cells, which align along the ECM fibres on the top of which mesenchymal cells also move when necessary. Mesenchymal migration is driven by forces generated at the leading edge, which relies heavily on: (a) the growth and retrograde flow of actin filaments, and (b) the activity of the mechano-enzyme myosin II are essential [6]. When cytoskeleton retrograde forces dominate adhesive tension at the leading edge, the cells are propelled forward.

Mesenchymal migration is however not ubiquitous. Several studies have shown that numerous cell types including T cells, neutrophils, dendritic cells and some tumour cells migrate in three-dimensional environments in the absence of ECM adhesion, instead using the ECM fibres as rigid support from which they propel themselves [7]. This is achieved via the protrusion of membrane extensions in the interstices of the ECM, and the pressure thereon can generate a sufficient tensile strength to induce the movement of the cell, typically referred to as amoeboid. This tensile strength is very low compared to that needed to propel the cell mesenchymal fashion, suggesting that amoeboid motility is more efficient and consumes less energy than mesenchymal migration [6]. Amoeboid migration is largely dependent on contractility of the cell under the control of Rho/ROCK and myosin II [8], and is characterised by the production of membrane blebs, dynamic pseudopod protrusion, cell retraction and rapid crawling. Blebs are spherical and short extensions of plasma membrane lacking actin and have a very short lifetime (up to a few seconds). Local detachments of the membrane due to the cortical tension of the actin cytoskeleton initiate the formation of blebs [9]. The detached membrane then expands by effect of the local intracellular pressure, causing the bleb to grow [10,11]. It was recently shown that increasing mechanical resistance of the environment increases blebbing rate [12]. During cell migration in a 3D environment, the bleb insinuates into the pores of the ECM, thereby polarizing the cell. The F-actin–denuded membrane of a newly formed bleb is an excellent template for actin polymerisation, which rapidly restores the cortex. In many cases, actin polymerisation continues after the cortex has been restored, transforming the bleb into a pseudopod [13]. The pseudopod then orients and guides whole-cell movement under the action of propulsive forces generated by the contractility of myosin molecular motors [6,14–16]. To summarise, a cell adopting an amoeboid migration mode rapidly adapts to the environment changes by forming protrusions and deforming throughout the crevices of the ECM without establishing a strong bond with the substrate, and therefore does not require local proteolysis of ECM fibres.

The two migration modes can coexist. For instance, some invasive cancer cells have the ability to degrade the ECM by expressing matrix metalloproteases at their surface and hence adopt a form of mesenchymal migration (phenotype hairpin, clustering of β1-integrin, degradation of extracellular matrix). However, after chemical inhibition of their extracellular proteases, these cells are able to compensate by adopting a proteolytic-blocked, amoeboid profile (rounded phenotype, travel by propelling the cell body, low adhesion) and continue their invasion of the ECM [17].

1.1. *The case for* Entamoeba histolytica

The amoeba parasite *Entamoeba histolytica* is the causative agent of amoebiasis, which includes invasion and destruction of the human intestine (causing dysentery) and liver (causing abscesses) [18]. The mechanism of amoeboid motion and the role of cell motility sustaining the invasion of human tissues by this parasite remain largely unknown. The questions raised to understand parasite motility in the context of infection are closely related to those raised for motile immunocompetent cells, which like *E. histolytica*, are activated during the inflammatory response and move in different environments such as blood, mucus, epithelia and lymphatic circulation. Like other motile cells, amoebic motility relies on

transient, diffuse adhesions [10], while the motor protein myosin II generates the traction forces required for efficient cell displacement [19].

To invade the intestinal tissue, this microorganism (a commensal of the colon) undergoes activation and then penetrates the mucosa. Although the signals that trigger this conversion are still totally unknown, two key factors appear to be essential: (i) polarisation of *E. histolytica* cells and (ii) motility of the amoebae directed by signals from the intestinal environment. During infection, the early inflammatory response of the host may constitute a signal enhancing parasite motility, and *in vitro* studies have shown that tumour necrosis factor (a pro-inflammatory molecule secreted during amoebiasis) attracts the amoebae [20].

Cellular and molecular *in vitro* studies have shown that *E. histolytica* motility occurs in two ways: *random motion*, mainly viewed as an environment-searching phenomenon, and *directed motion*, guided by chemotactic signals (*cf.* Fig. 1). Random motion is characterised by the formation of blebs caused by the disjunction of the plasma membrane from the actin cortex under the effect of an increased turgor pressure [10]. Directed migration is driven by cell polarisation via the formation of a unique pseudopod at the front and a uropod at the rear of the cell, the latter being characterised by high densities of ligand–receptor complexes and adhesion molecules that patch and cap at the amoebic surface [21]. Uropods are also formed by neutrophils, monocytes, and natural killer cells, where they play important roles in cell adhesion during immune interactions. To summarise, the motility of *E. histolytica* motility requires: (i) polarisation of the cell via the protrusion of a pseudopod enriched in actin and partner proteins such as myosin IB [22], ABP-120 [23] and p-21-activated kinase [24]; (ii) adhesion of the pseudopod to the substrate and iii) contraction of the cell body. It has been shown that the PAK protein, enriched at the migration front, regulates the formation of pseudopodia [24,25]. Protein ABP-120, capable of binding actin, is also located in the pseudopodia during migration, and interacts with a membrane lipid, sulfatide, suggesting a role in the stabilisation of the actin cortex during protrusion of the pseudopodia [26].

Displacements (but not bleb formation) of *E. histolytica* requires adherence to the substrate on which it operates. A membrane receptor of 140 kDa similar to integrin β1 has been identified. It is capable of forming a molecular signalling complex leading to the formation of adhesion complexes involving FAK, the paxillin and vinculin [27]. The filamentous actin is localised along the lateral sides of the trophozoites, and is indispensable to the maintenance of cortical tension of the cell body [28], but also at the rear of the cell, in association with myosin II. Propelling the cell body is caused by the contraction of the uropod where myosin II fulfils its role of molecular motor [19]. The application of an intracellular force (using magnetic beads) does not affect cell polarisation and migration, whereas the application of the force at the rear of the cell induces a persistent polarisation and strongly directional motion, almost directly opposed to the magnetic force [29]. This phenomenon is completely abolished when phosphatidylinositol 3-kinase (Pi3K) activity is inhibited by wortmanin, demonstrating that the applied mechanical stimulus was transduced and amplified into an intracellular biochemical signal, a process that allows such low-intensity force to strongly modify the migration behaviour of *E. histolytica*.

In the presence of gradients of chemoattractant molecules such as bacterial or erythrocyte lysates, anaphylatoxin C5a, the N-terminal portion of fibronectin, N-acetylneuraminic acid, Tumour Necrosis Factor (TNF) and Interleukin-8, the trophozoites are oriented and induce a sustained movement towards the source [20,30–32]. It is believed that induction of chemotactic migration by ECM proteins or inflammatory factors is an important process in the pathophysiology of amoebiasis, causing the invasion of the