Barotaxis: how cells live and move under pressure

Ana-Maria Lennon-Duménil, Hélène Moreau

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Ana-Maria Lennon-Duménil[1] and Hélène D. Moreau[1]

Addresses
[1]Institut Curie, PSL Research University, INSERM U932, F-75005 Paris, France

Corresponding author: Hélène D. Moreau (helene.moreau@curie.fr)

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Abstract

Cell migration is an essential process that controls many physiological functions ranging from development to immunity. *In vivo*, cells are guided by a combination of physical and chemical cues. Chemokines have been the center of attention for years, but the role of physical properties of tissues has been under-investigated. This despite the fact that these properties can be drastically modified in pathology. Here, we discuss the role of one important tissue physical property, hydraulic resistance, in cell guidance, a phenomenon referred to as barotaxis, and describe the underlying physical principles and molecular mechanisms. Finally, we speculate on the putative role of barotaxis in physiological processes involving immune and cancer cells.

Introduction

Cell migration is an essential process in the life of many organisms. On one hand, unicellular organisms such as *Dictyostelium discoideum* may use migration for feeding or finding more favorable environments. On the other, pluricellular organisms such as mammals rely on cell migration not only for their development, but also for their survival, as cell migration is instrumental for the action of immune cells that circulate between tissues and lymphoid organs. However, cell migration can also be detrimental and lead to pathology as it enables cancer cell spreading and invasion of healthy tissues. Unraveling the cell-intrinsic molecular mechanisms underlying cell locomotion as well as their regulation by environmental cues is thus essential to understand cell migration-dependent physiological and pathological processes [1-4].

The molecular mechanisms underlying single cell migration are highly conserved across eukaryotic cells. They can be divided is two main categories: (1) amoeboid migration [5] and (2) mesenchymal migration [3]. In both migration modes, forward movement relies on the actin cytoskeleton. In amoeboid migration, the actin network contracts the cell rear via its molecular motor myosin II, whereas, in mesenchymal migration, the actin cytoskeleton allows formation of protrusion at the cell front [6]. Remarkably, cells can switch from one migration mode to another depending on the adhesiveness and degree of confinement imposed by the environment: while low adhesion under high confinement favors the amoeboid migration mode, strong adhesion and low confinement stimulates mesenchymal migration [5, 7]. Additional chemical (chemokines, growth factors…) and physical (stiffness, topography…) cues present in tissues can influence cell migration by impacting cell speed and/or modifying cell guidance through diverse mechanisms [4, 8, 9].

More recently, hydraulic resistance, i.e. the force that surrounding fluid opposes to cell migration, has arisen as an important parameter determining cell directionality [10], a phenomenon called barotaxis (for cell guidance by pressure). Here, we will review our current understanding of the molecular mechanisms underlying this process, as well as how cells prioritize distinct cues to adopt defined migration patterns. We will finally highlight how barotaxis may contribute or impede cell function and discuss its relevance in various physio-pathological contexts.

Barotaxis: cell guidance by surrounding fluid forces

In order to move, cells have to displace the surrounding fluid. In unconfined settings, fluid flows around the migrating cell, and hydraulic resistance is thus not generated, although shear stress and frictional forces may be produced and impact cell migration. However, in confined environments, cells need to push the fluid in front of them in order to move forward, hence generating hydraulic resistance, which is proportional to the column of fluid the cell has to displace (**Box 1**).

Barotaxis was first revealed using microfluidic devices presenting bifurcations [10] (**Box 1**). Neutrophils (HL-60 cell line) facing bifurcations of distinct hydraulic resistance tend to choose the path of least resistance (smallest column of fluid to displace). They

exhibit a gradual barotactic response, being more biased toward low resistance path when the resistance difference between both paths is bigger.

Importantly, to be barotactic, cells need to generate hydraulic resistance themselves, by pushing the fluid. This is the case for confined neutrophil-like cells that push fluid in front of them as demonstrated by the displacement of small beads in front of them [10] (**Fig. 1A, top**). However, cells that have the capacity to transport the fluid in front of them by another mean than by pushing it might not generate such resistance and be insensitive to barotaxis. This has been observed in immature dendritic cells, i.e. the dendritic cells that are in charge of patrolling peripheral tissues [11]. For this, immature dendritic cells continuously sample their microenvironment by ingesting surrounding fluid while migrating. Fluid ingestion occurs through macropinocytosis, an actin-dependent process that allows cell that allows cells forming giant vesicles from membrane ruffles [12, 13]. Macropinocytosis enables immature dendritic cells to efficiently transfer fluid from the cell front to the cell rear as these liquid-loaded vesicles that form at the cell front are then secreted at the back of the cell (**Fig. 1A, middle**). Therefore, this process renders immature dendritic cells insensitive to hydraulic resistance and endows them with the ability to explore environments exhibiting theoretical infinite hydraulic resistance such as dead-ends. In contrast, mature dendritic cells lose their capacity to perform macropinocytosis, regain a barotactic behavior and migrate directionally toward lymph vessels, carrying the antigens captured in peripheral tissues to lymph nodes. Barotaxis may help these cells avoiding dead-ends and choosing the path of low hydraulic resistance, facilitating their arrival to lymph nodes for antigen presentation to T lymphocytes and rapid initiation of adaptive immune responses.
Interestingly, a trend to barotaxis has also been observed in *Dictyostelium discoideum*, although with decreased sensitivity to hydraulic resistance difference (at least an 8-fold difference in hydraulic resistance is needed). Hence, *D. discoideum* appears globally less biased by hydraulic resistance as compared to neutrophils or non macropinocytic dendritic cells, which respond to a 4-to-5-fold difference [10, 11, 14]. In particular, some cells within the population were able to explore dead-ends. Yet, in these cases, the authors indicate that these specific cells were either smaller than the cross-section of the channel or deformed enough to allow fluid flow around them, preventing hydraulic resistance generation (**Fig. 1A, bottom**), with no evidence for macropinocytosis involvement in fluid transport across the cell. These observations confirm that barotaxis would be the general rule for any cell migrating in confinement with no other choice than pushing the surrounding fluid. Of note, barotaxis is not restricted to fast-moving cells. Indeed, barotaxis has recently been reported in very slow migrating cancer cell lines (namely blebbing MDA-MB-231 breast cancer cells and HT1080 fibrosarcoma cells [15]), which display a strong sensitivity to subtle hydraulic resistance differences (about 2-fold difference in hydraulic resistance is sufficient to bias the cells).

Box 1: Microfluidic tools to study barotaxis

Barotaxis can only take place in confined environments, where cells are pushing the fluid in front of them to progress, and hence, are generating hydraulic resistance that

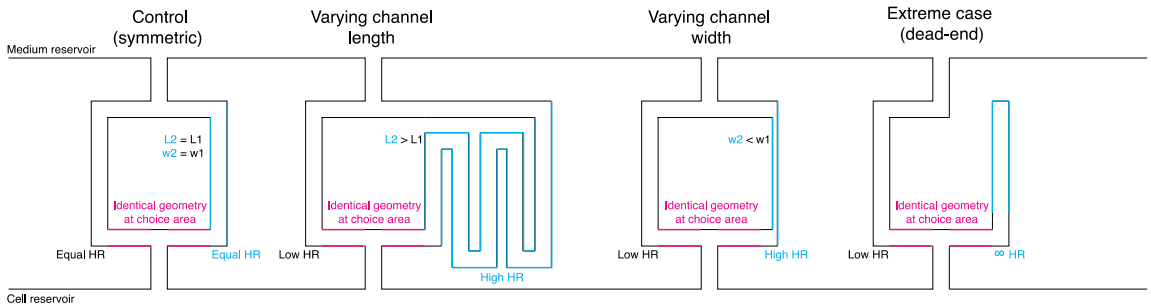
can then guide them. A barotactic choice of direction happens when cells are facing bifurcations of two or more paths that exhibit different hydraulic resistance.

The hydraulic resistance is determined by both the length and the cross-section of the column of fluid that the cell pushes while migrating according to equation (1) for a rectangular section:

$$R_h = 12\mu L / (wh^3 (1-0.63h/w)) \quad \textbf{[1]}$$

With w width of the channel, L its length, h its height, and μ the dynamic viscosity of the fluid [14, 16].

The experimental model of choice to study barotaxis is microfluidic devices [17]. Typically, cells are placed to migrate in microchannels in which they are confined, filling the full cross-section of the channel, avoiding fluid leakage and enabling the cell migration-dependent generation of hydraulic resistance. While migrating, the cell encounters a bifurcation (or more branches) that forces it to make a directional choice. The channels after the bifurcation are designed (length, width) to exhibit different hydraulic resistances [10]. Importantly, the change in height or width should happen not at the bifurcation site, but a few microns away from it, so that the cell chooses its direction based on hydraulic resistance only, rather than based on confinement variations (in particular of the nucleus [18]). Of note, the angle of the bifurcation is predicted not to have any impact on barotactic choices (Blanch-Mercader, Voituriez, Piel, Moreau, unpublished).



More complex devices can of course be designed to address more complex questions, such as: how a cell will behave facing successive bifurcations in a maze? How barotaxis compete with chemotaxis? [4, 17, 19]

Molecular mechanism underlying barotaxis

As highlighted by all the studies done so far, one imperative criterion for barotaxis to occur is for the cell to be completely confined and impermeable to fluid, so that it has to push the fluid in front of it in order to move [10, 11, 14] (**Fig. 1A, top**). If fluid can freely flow around or across the cell, then no hydraulic resistance is generated, and barotaxis cannot exist. However, if the fluid impermeability criterion is fulfilled, then barotaxis appears to be a conserved process enabling migrating cells to choose the path of least resistance.

The first report on barotaxis [10] described the directional choice as a competition between extending arms, an observation confirmed in later reports, regardless of the cell type and migration mode (in *D. discoideum*, neutrophils, dendritic cells, cancer cells…). When facing a bi- or a trifurcation, cells extend protrusions, first at similar rates, until one arm takes the lead and expands faster, leading to the rapid retraction of the losing arm [10, 11, 14, 15] (**Fig. 1B**).

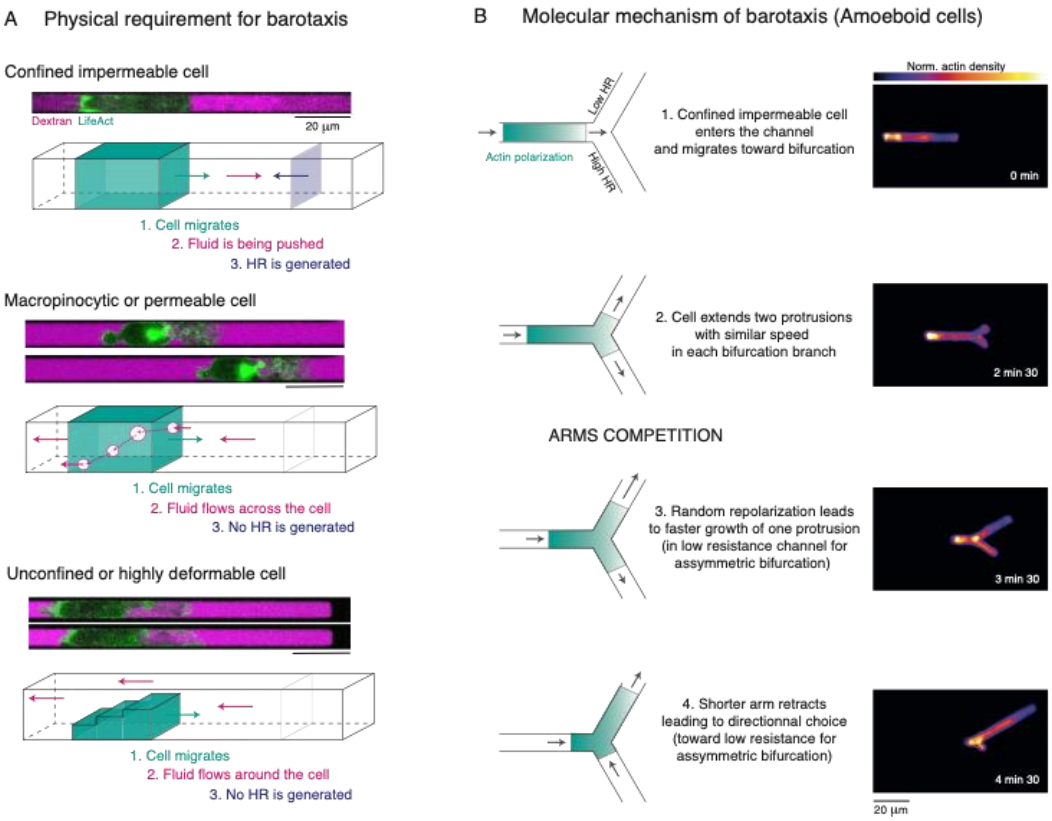


Figure 1: *Mechanism of barotaxis.* **(A)** Physical requirement for barotaxis. To be subjected to barotaxis, cells need to generate hydraulic resistance in front of them. This only occurs for confined cells that completely block the cross-section of the channel and have to push the fluid to move forward (**top panel**). Cells that are able permeable to fluid (macropinocytic cells, **middle panel**) or not fully obstructing the channel cross-section (unconfined or highly deformable cells, **bottom panel**) are not generating hydraulic resistance while migrating and are therefore not susceptible to barotaxis. **(B)** Mechanism of barotaxis for amoeboid cells. (Adapted from Moreau et al, Dev Cell 2019).

But how does this competition between arms occur and what does determine its outcome? To understand the mechanisms underlying competition, it is essential to keep in mind that the differences in hydraulic resistance only generate very low opposing forces to migration. Therefore, sensing of hydraulic resistance is only compatible with cells that exert mild forces on the substratum such as immune cells, *D. discoideum* and other cells that use the amoeboid mode of migration [5, 6, 11, 20, 21]. In contrast, hydraulic resistance sensing, and thus barotaxis, is not compatible with cells undergoing adhesive migration as they generate forces on the substratum that are several orders of magnitude above hydraulic resistance forces [20]. Adhesive migration could therefore represent a mechanism for cells to escape barotaxis.

Theoretical modeling combined with experimental data [11] demonstrated that barotaxis results in fact from the force imbalance generated by hydraulic resistance, but is essentially a passive phenomenon from the point of view of the cell, not requiring any

specific receptor or signaling pathways. In this model, acto-myosin front/back polarity is a key determinant in "making the right choice": the more a cell is polarized when reaching the bifurcation, the more chances it has to choose the low resistance path. The cell extends two arms, at similar speed, forming an upper system composed of the two arms only, which randomly polarize and finally move towards one direction once it reaches a certain threshold. This threshold corresponds to the force needed to counteract hydraulic resistance and move forward. It is thus reached faster toward the low resistance side. It was proposed from this work that acto-myosin cytoskeleton amplifies the small force imbalance created by hydraulic resistance, hence inducing barotaxis.

This mechanism would apply to any migrating cell exhibiting acto-myosin accumulation at the cell rear and is compatible with the observations made in amoeboid cells such as neutrophils [10] and *D. discoideum* [14],with accumulation of myosin in the uropod and retracting arm. Interestingly, the weaker polarization of actin observed in *D. discoideum* could explain its lower sensitivity to hydraulic resistance. Of course, this mechanism, although theoretically universal for polarized migrating cell, does not exclude modulation of barotaxis by intracellular signaling pathways regulating acto-myosin cytoskeleton dynamics. Interestingly, a similar mechanism was proposed for chemotaxis [22].

Similar behavior is observed in blebbing cancer cells, with arms protruding in the different branches first at similar speed, then one growing faster, taking the lead and imposing choice of low hydraulic resistance [15]. Lower actin content was detected in the winning arm while higher myosin was detected in the losing arms (and rear of the cell). In these cells, barotactic behavior was dependent on local calcium signaling through the stretch-activated channel TRPM7, which is turned on by hydraulic resistance through membrane tension and induces a local thickening of the cell cortex. Of note, these slowly migrating cells have been reported to use a water-driven migration mechanism rather than a classical actin driven one under strong confinement [23], and recent modeling has demonstrated the critical role of hydraulic resistance in driving that type of migration [24, 25].

Cell interpretation of hydraulic resistance among other guidance cues

In simple highly controllable *in vitro* environments, hydraulic resistance can be isolated as a single parameter. Yet in complex natural environments, cells constantly deal with multiple cues, coherent or contradictory, and they must integrate these different signals in order to determine their polarization and migration direction [1] (**Fig. 2**).

Hydraulic resistance occurs in natural contexts when cells such as neutrophils are migrating in small vessels, or when cells such as patrolling dendritic cells are confined in tissues bathed in interstitial fluid [11, 26]. Importantly, hydraulic resistance has the particularity of being an integrated cue over the whole path of cell migration. That is, while many guidance cues are detected locally by migrating cells (chemokine concentration, local stiffness, porosity…), the hydraulic resistance sensed by the migrating cell is the one generated by the whole column of fluid the cell is pushing in front of itself. This means that, in a maze, cells would be able to determine the overall lowest resistance path. Modeling of barotactic and non-barotactic cells in mazes

supports this idea [11]. Interestingly, barotactic cells identify the low resistance path and rapidly exit a network of channels. On the contrary, non-barotactic cells (e.g. macropinocytic but also highly deformable or unconfined cells) are better at exploring the complete network. This exploration is key for immature dendritic cell function that is to efficiently detect harmful signals in the confined environment of tissues, a property confirmed by *in vivo* imaging [11].
The function of neutrophils is fundamentally different than the one of dendritic cells as, rather than patrolling the environment, these cells must be recruited as efficiently as possible to sites of infection. Therefore, avoiding dead-ends, or highly resistant paths might help neutrophils reach rapidly their site of action. Amazingly, this has been very recently reported, not only as individual cells [10], but rather as a community behavior [26]. *In vivo* imaging of neutrophils in capillaries revealed that neutrophils following each other in the network tend to alternate direction choices at bifurcation, avoiding jamming. This particular behavior was further confirmed *in vitro* in microfluidic designs, unravelling a double mechanism that optimizes the alternance of choices: the first neutrophil (i) augments the hydraulic resistance of the channel wherein it migrates, inducing a bias in the migration of the following neutrophil, which then choses the other channel (lower hydraulic resistance); and (ii) also prevents chemokine diffusion, locally modifying the chemotactic gradient and reinforcing the choice of the second neutrophil toward the neutrophil-free low resistance path.

The interplay between cells and different cues in complex tissue environments is therefore likely to modulate how migrating cells are interpreting combinations of guidance signals. So far and to our knowledge, only chemotaxis has been tested in competition against barotaxis. While barotactic signals are dominant in neutrophils [10], chemotactic signals govern *D. discoideum* migration [14]. This could be explained (i) by the differential sensitivity of neutrophils and *D. discoideum* to barotaxis, and (ii) by the range of hydraulic resistance differences and concentrations/gradients of chemokine tested. The molecular pathways implicated in chemotaxis and barotaxis appear to be distinct. Therefore, while it is clear that different cells may prioritize differently the distinct guidance cues they are facing, it is difficult to predict cue competition outcome and draw a general rule. Future studies will help deciphering how cells interpret multiple signals and how these signals determine their pattern of migration and behavior within tissues.

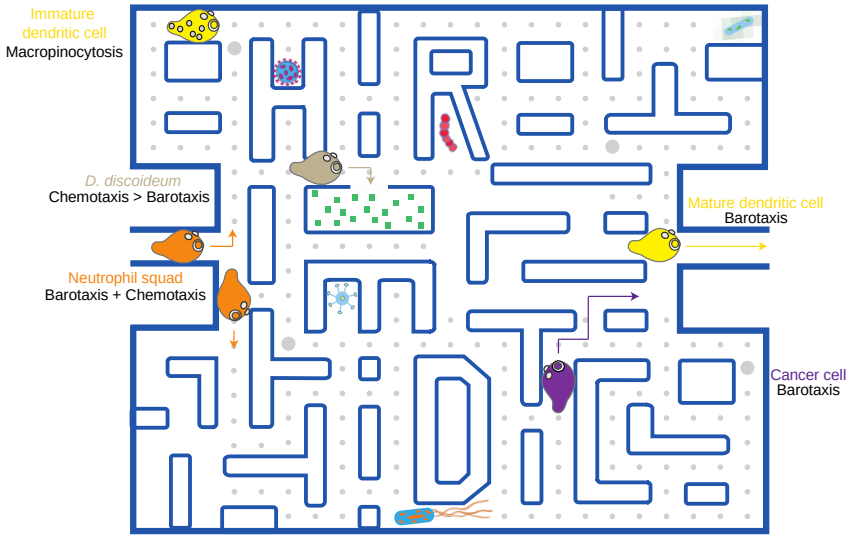


Figure 2: *Integration of environmental cues during migration and impact on cell function.* The complex environments in which cells are migrating provide multiple guidance cues, in particular heterogeneous hydraulic resistance or chemokine distribution. Immune cells have a differential susceptibility to these guidance cues. While immature dendritic cells are insensitive to hydraulic resistance thanks to macropinocytosis and tend to fully explore a maze, mature dendritic cells have lost this capacity and rapidly find the exit thanks to barotaxis. Of note, mature dendritic cells are also guided by the CCR7/CCL21 chemokine axis, although competition between chemotaxis and barotaxis has not been tested yet. Barotaxis dominate in neutrophil guidance, and not only plays a role for single neutrophils but also for neutrophil squads, enabling them to avoid jamming of capillaries. On the contrary, chemotaxis guidance is dominant for the amoeba *D. discoideum*. Finally, at least some cancer cells are barotactic. Whether turning on macropinocytosis to fulfill metabolism needs lowers the barotactic sensitivity of cancer cells and enables them to reach hidden metastatic niches remains to be investigated.

Conclusion and perspectives

In conclusion, we here review the growing literature on the key role played by hydraulic resistance in guiding cell migration when confined in tissues. These studies suggest that barotaxis has emerged early in evolution, as it is conserved from amoebas like *D. discoideum* to immune cells. Remarkably, it appears that these distinct cell types can use specific cell-intrinsic and -extrinsic mechanisms to either avoid or take advantage of directional biases imposed by hydraulic resistance, such as macropinocytosis in dendritic cells or "follower-exclusion" in neutrophils. Future studies shall now focus on defining the physiological contribution of barotaxis to pathology *in vivo*, for example in the context of cancer metastasis or adaptive immune responses.

Conflict of interest statement

None declared.

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Author contribution

Conceptualization: HDM. Writing – Original draft: HDM. Writing – Review & editing: AMLD and HDM. Visualization: HDM. Funding acquisition: AMLD and HDM.

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