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Running title: Polarity in immune cells

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Abstract

Immune cells are responsible for pathogen detection and elimination, as well as for signaling to other cells the presence of potential danger. Regulation of cell polarity controls different aspects of immune cell behavior: migration, cell-cell interactions, and division. This review aims at providing an overview from both biology and physics perspectives of how cell polarity thereby shapes immune cell function.

Keywords (5-10 words): Immune cell, cell polarity, polarization, migration, immune synapse, asymmetric cell division

1. Introduction

The immune system defends the organism from pathogen invasions, infections and, more generally, any pathological situation (for example cancer). Once a pathogen has gone through the first line of mechanical defense of epithelia such as skin and gut or lung mucosae, it encounters patrolling and

sentinel cells such as dendritic cells, macrophages, and neutrophils. These cells eliminate the pathogen or transport a piece of it (antigens) to the closest lymph node where they interact with other cell types (lymphocytes). This step, referred to as antigen presentation, initiates the adaptive immune response.

In lymph nodes, lymphocytes patrol the micro environment in search of antigens. In particular, naive $CD8^+$ T cells *migrate*, and when they encounter an antigen presented by antigen-carrying cells (such as dendritic cells) they form a cell-cell contact called *immune synapse*. Following this encounter, they get activated (able to kill an infected cell) and divide several times producing effector and memory progeny with different functions, through *asymmetric cell division*. Effector cells *migrate* back to the tissue to kill target cells via cytotoxic *immune synapses*. Ultimately, while most of these cells die, a small population of long-living cells remains in the organism as memory cells, to respond to a second infection. These three functions of $CD8^+$ T cells (migration, cell-cell contact, and asymmetric cell division) are common to several immune cells, for example, dendritic cells also patrol the tissue and present the antigen to $CD4^+$ and $CD8^+$ T cells at an immune synapse. B cells scan (by migrating) the subcapsular sinus of the lymph node to acquire native unprocessed antigen from macrophages through an immune synapse and then migrate to the T cell zone to present the antigen on the MHCII before differentiating (after several rounds of affinity maturation) in antibody-producing cell and memory cells.

Cell polarity is defined as the capacity of a cell to displace substructures and molecules in a specific place defining an axis of symmetry to accomplish a biological function. The three functions described above require the establishment of a precise polarity axis: (1) when a cell migrates, cytoskeleton components (actin and myosin) and adhesion molecules are asymmetrically distributed; immune cells can migrate by different mechanisms, requiring or not adhesion, and therefore, they can use different types of polarity to move. (2) The formation of the immune synapse is accompanied by the establishment of a polarity axis passing through the center of the lymphocyte and the cell-cell contact. (3) In some cases, the polarity axis defined in this way allows cells to divide and give rise to daughter cells with different phenotypes (asymmetric cell division).

In most tissues, cells acquire a polarity axis to identify a side of the cell as ‘external’ or ‘apical’ and this is maintained for the whole cell life. Cell polarity in immune cells is adapted to provide orientation in motility, synapse formation/stability, and asymmetric cell division on demand: it is more “flexible” than tissue polarity as cells must have the possibility to form and maintain a single stable polarity axis for hours but must be able to quickly break up this axis and re-polarize within minutes.

In this review, we will provide an overview of the role of cell polarity in immune functions: instead of listing any molecular pathway involved in cell polarity, we will focus on the biological context where immune cells polarize to fulfill their functions.

2. Cell polarity and immune cell migration

The first immune cell function where polarity is crucial is motility. Cells move, *in vivo*, by two main types of migration: mesenchymal (protruding an adhesive lamellipodium and retracting the back forward in an inchworm movement), amoeboid (producing a contractile region at the back and advancing thanks to cortex pressure gradient) (See Fig.1). In all cases, cells reorganize their internal components along the front-back axis during migration: in mesenchymal migrating cells, the centrosome and Golgi apparatus appear in front of the nucleus, while in amoeboid migrating cells the nucleus precedes the centrosome and the Golgi apparatus (Luxton & Gundersen, 2011). In mesenchymal migrating cells, actin polymerization is promoted, in particular at the lamellipodium, through the Cdc42/Rac pathway. A decreasing back/front gradient of RhoA (opposite to the Rac pathway) enhances contraction at the back, in particular in amoeboid migration where the uropod, a strong contractile unit, enriched in actomyosin, ezrin, and ROCK kinase (Sánchez-Madrid & Serrador, 2009), propels the cell (Hind, Vincent, & Huttenlocher, 2016). Evolutionarily conserved polarity proteins such as Scribble and Dlg are polarized at the rear end of the cell, while Par3 is concentrated at the front (Krummel & Macara, 2006).

Independently of the motion type, migrating cells require friction to transfer forces to the substrate and advance. In mesenchymal migration, this friction is provided by integrins polarized at the front

(See Fig.1). For example, to move along the wall of a blood or lymphatic vessel, a T cell requires adhesion through LFA-1/ICAM-1 interaction. When a T lymphocyte needs to exit a vessel (extravasation or diapedesis) it also does so by anchoring itself through specific integrins to the tissue and squeezing between two cells (or even inside a cell) to extravasate. Blocking LFA-1/ICAM-1 interaction results in a lower number of T cells in the lymph node, suggesting that this type of movement (along the wall of a vessel and the extravasation) relies on integrins (Teijeira et al., 2017). In this type of motility, cells present a peculiar, polarized shape with a large lamellipodium at the front (where integrins are concentrated).

Most immune cells migrating in 3D, for example in a dense collagen network, use an amoeboid type of migration. In this case, the motion is provoked by actomyosin contractions at the back, and the friction is provided by pushing on the surrounding cells (R. J. Hawkins et al., 2009) (See Fig.1). The role of integrins depends on the environment and the type of motility. It has been shown that integrin-dependent adhesion is dispensable for amoeboid motion (Lämmermann et al., 2008). Actin polymerization at the front or along the side of the cell contributes to friction and propulsion. These mechanisms can be finely tuned by a signaling network. For example, it has been shown that, in migrating T cells, chemokine receptors (such as CCR7) control cortical actin flow independently of the integrin pathway: while integrins regulate friction, chemokine receptors modulate the location and the speed of actin polymerization and myosin II contraction (Hons et al., 2018). This provides the cell with a mechanism to turn on when following a chemotactic gradient.

In 3D environments, it has been shown that knocking out integrins and chemokine receptors reduces the migratory speed of T cells by no more than 30%, indicating that there are other mechanisms providing residual friction. In fact, cells can use tissue topography to move even in absence of integrins (Reversat et al., 2020), literally grasping extracellular matrix fibers. In the case of neutrophils, this has been shown to be a WASP-mediated mechanism: WASP sorts to sites of saddle curvature and links topography to actin polymerization (Brunetti et al., 2022).

In moving cells, the stability of the polarity axis defines the persistence of the motion. In a cell race where many cell types were put in competition on fibronectin stripes, an unexpected relation between persistence and speed has been uncovered: the fastest cells were also the ones that changed direction less frequently (Maiuri et al., 2012). This observation, far from being intuitive, has been confirmed in 2D and *in vivo* and explained with a model that links the robustness of polarity with the speed of the cells (Maiuri et al., 2015). In this model, polarity is defined by “polarity cues”, molecules organized in front/back (or back/front) gradient, responsible for actin flow and cortex contractility. The specific molecule depends on cell type: Myosin II, regulators of actin and microtubule polymerization, etc, hence for the speed of the actin flow and ultimately for the cell speed. For example, in dendritic cells the accumulation of contractile molecular motors at the back increases speed and reduces the chance of changing direction arising from noise (making the polarity axis more robust) (Chabaud et al., 2015).

Migration in confined environments has been conveniently studied *in vitro* in microchannels. Using this system, for example, it has been shown that naive dendritic cells, in charge of patrolling tissue in search of antigens, alternate fast and persistent motility (well polarized) phases with slow diffusive phases during which they uptake large lumps of surrounding fluid (a process called macropinocytosis) (Faure-André et al., 2008). While moving fast (and persistently), they asymmetrically displace myosin II to the cell rear. When they pause, this molecular motor, responsible for cortex contraction, is recruited to the front where the cell needs it to form large macropinosomes (Chabaud et al., 2015): this reflects a transient change in cell polarity. Another modification of (actin) polarity occurs when the cell passes through a constriction: actin is recruited around the nucleus, in an Arp2/3-dependent mechanism, to induce transient nuclear lamina rupture (Thiam et al., 2016). Of note, the nucleus of a cell moving in a confined environment, a dendritic cell, for example, acts as a mechanical gauge. When the cell is moving, this organelle is at the front of the cell: the diameter of the nucleus enables the cell to discriminate pore size and effectively choose the path of least resistance; when the centrosome has passed the constriction, it signals nuclear passage to the trailing edge of the cell,

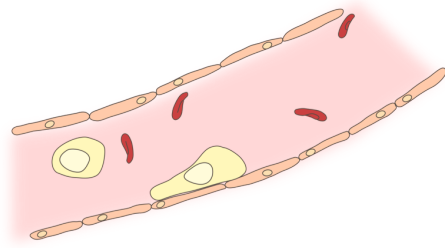
effectively coordinating the movement of the whole cell through the hole: proper polarity is required for cell migration in complex environments (Renkawitz et al., 2019).

While using the amoeboid mode, dendritic cells, neutrophils and T cells maintain the centrosome behind the nucleus, with myosin II at the cell rear to propel the cells through a complex environment. The integrity and positioning of the microtubule network are crucial to organize the actomyosin machinery: indeed, it has been shown in dendritic cells and neutrophils that the presence of microtubules at the back releases GEF-H1, a guanine exchange factor of RhoA, which is a classical activator of formin-dependent actin polymerization and ROCK-dependent myosin II contraction (Kopf et al., 2020).

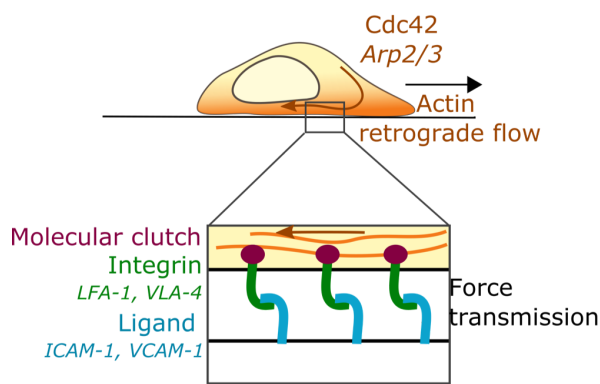
Migrating immune cells explore any hidden corner of the tissue or closed spaces. In confined spaces, they can block the passage of fluid with their own body, resulting in an increase in hydrostatic pressure. By sensing the increase in pressure, cells are able to migrate in a complex environment choosing the path of least resistance, a property named barotactism. Polarity is necessary for the cell to be barotactic: in fact, the more asymmetric actin distribution is along the axis, the more the cell is able to sense pressure difference. This property allows, for example, activated dendritic cells to reach the closest lymph node once they have encountered enough antigen in the tissue. Immature dendritic cells have a different polarity (i.e. different actin distribution) and are not barotactic, as they overcome hydrostatic pressure by engulfing fluid at their front and discarding it on their side or at the back while doing macropinocytosis (Moreau et al., 2019).

Non-confined environment

example: leukocyte in blood vessel



adhesion-dependent

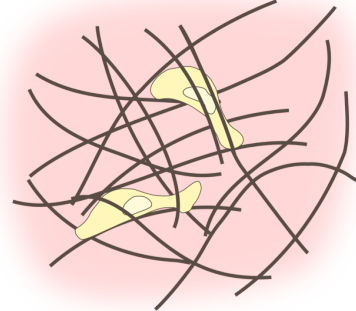


Fast flow - Fast migration
Slow flow - Slow migration

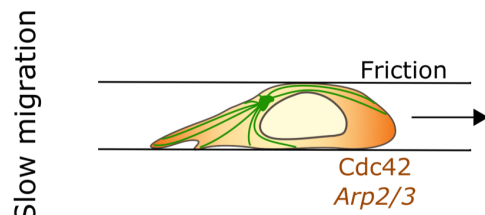
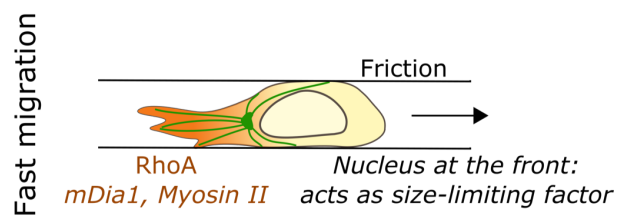
Flow regulated by:
Balance force transmission vs F-actin release
Actin polymerization/depolymerization

Confined environment

example: leukocyte in tissue



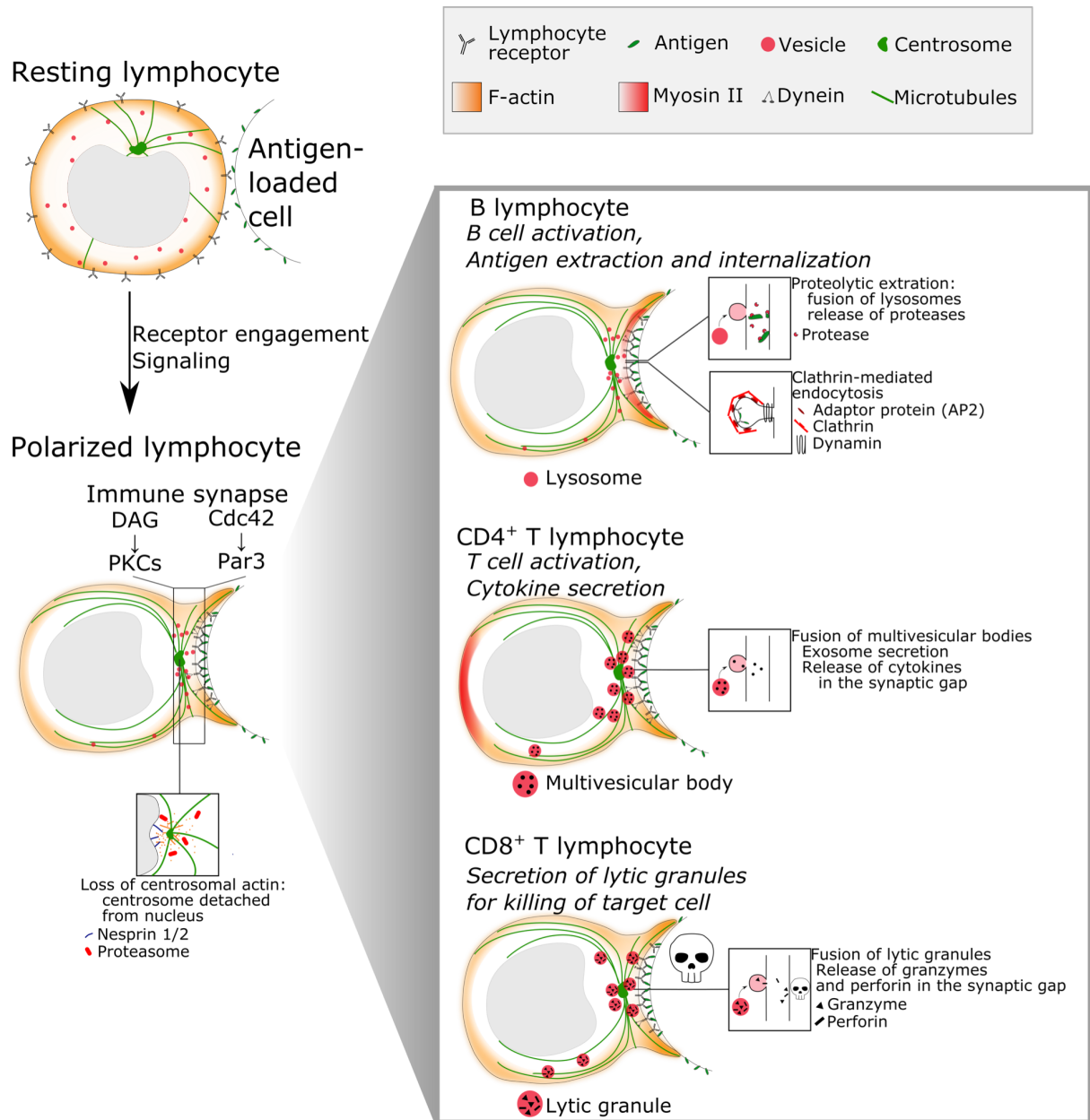
adhesion-independent



[Insert figure 1] Figure 1: Two main types of motility in immune cells. Cells in open environments tend to migrate by a molecular clutch mechanism, where friction is regulated by integrins. Cells in confined environments tend to migrate by amoeboid motion, generating a gradient of contraction in their cortex and regulating adhesion by integrin-independent friction.

3. Polarity at the immune synapse

3.1 Polarized reorganization of organelles at the immune synapse



[Insert figure 2] Figure 2: Polarity at the lymphocyte immune synapse. Left: general processes described in all lymphocytes. Right: Specific mechanism in the three main classes of lymphocytes.

Immune synapse and polarization. After a phase of motility, immune cells stop, generally because of an interaction with another cell. In this contact, the antigen is recognized and the cells form an immune synapse, a signaling platform that allows intercellular communication and determines the fate of the cells. This structure was first described in T lymphocytes but has now been observed in many different lymphocytes/antigen-loaded cell encounters. The immune synapse serves as a platform for antigen affinity discrimination, receptor-mediated signaling, and cell-cell communication through exocytosis and endocytosis events. The formation of this cell-cell junction is accompanied by the complete reorganization of lymphocyte's organelles, which align along an axis to polarize and serve the functionality of this junction. Mostly, this is characterized by the repositioning of the centrosome towards the immune synapse, recruitment of vesicles, F-actin polymerization, and rearward transport of the nucleus (see Fig.2). Centrosome polarization and actin remodeling appear to rely mostly on the conserved Par polarity complex, via Cdc42, and the small GTPase Rac (Yuseff et al., 2011) (Stowers, Yelon, Berg, & Chant, 1995), however, some studies question the role of Cdc42 in centrosome polarization of CD4⁺ T cells (Chemin et al., 2012; Gomez et al., 2007). Of note, apart from a study showing that B cells presenting antigenic peptides to CD4⁺ T cells reorient their centrosome and endocytic/exocytic machinery towards the immune synapse (reciprocal to the better-studied T cell side) (Duchez, Rodrigues, Bertrand, & Valitutti, 2011), very little is known about the existence of a reciprocal polarization of the antigen-presenting cell in general, and its potential role in immune synapse formation and function remains unclear.

Despite their similar final organization, immune synapses have widely different functions depending on the type of lymphocyte, resulting in some specificities, in particular regarding the types of vesicles polarized to the immune synapse. Naive T cells use the immune synapse to become activated, and for this purpose, they polarize TCR recycling endosomes towards the immune synapse, which allows naive T cells to maintain high TCR signaling for several hours, as required for their activation (Das et al., 2004; Iezzi, Karjalainen, & Lanzavecchia, 1998). In contrast, the resulting effector CD4⁺ T cells use the immune synapse to release cytokine-rich exosomes from multivesicular bodies, that are polarized to the immune synapse, in order to support the activation of the opposing cell, for example,

a B cell (Huse, Lillemeier, Kuhns, Chen, & Davis, 2006; Ueda, Morpew, McIntosh, & Davis, 2011). Effector CD8⁺ T cells are meant to kill their target cell by polarizing lytic granules at the immune synapse, to eventually release granzymes and perforins in the synaptic gap, resulting in the death of the opposing cell (Kupfer, Dennert, & Singer, 1983; Jane C Stinchcombe, Majorovits, Bossi, Fuller, & Griffiths, 2006). Finally, naive B lymphocytes use the immune synapse to become activated, and to extract antigen from the surface of the opposing cell, either by pulling on the antigen in a myosin II-dependent manner -mechanical extraction- or by releasing proteases into the synaptic gap by polarizing and fusing lysosomes with the synaptic membrane -proteolytic extraction-, eventually internalizing it into endosomes for further processing and cross-presentation to CD4⁺ T cells (Natkanski et al., 2013; Spillane & Tolar, 2017; Yuseff et al., 2011). This highlights the fundamental differences between these immune synapses and might explain their discrepancies both in terms of final organization and polarization processes.

Nucleus centrosome axis. Lymphocyte polarization at the immune synapse implies the formation of a nucleus-centrosome axis, corresponding to the lymphocyte/antigen-loaded cell axis. The bean-shaped lymphocyte nucleus takes up most of the cytoplasmic space, with the centrosome closely associated with the nuclear membrane and sitting in its groove (Pineau et al., 2022; Ulloa et al., 2022). Given the space constraint imposed by the nucleus during lymphocyte polarization at the immune synapse, research groups have started investigating its evolution during this process. Recent studies have shown that, in B lymphocytes, the nucleus reorients together with the centrosome to have its groove facing the immune synapse, is transported to the rear of the cell, and deforms to increase groove area (Pineau et al., 2022; Ulloa et al., 2022). There is increasing evidence for the crucial role of nucleus-cytoskeleton interactions in lymphocyte activation and immune synapse formation. Indeed, the regulation of myeloid cell nuclear shape appears to control cell fate both during hematopoietic differentiation and lymphocyte activation (Biedzinski et al., 2020; Fabrikant, Gupta, Shivashankar, & Kozlov, 2013; Gupta, Marcel, Sarin, & Shivashankar, 2012). Moreover, the LINC complex (Linkers of the Nucleus and the Cytoskeleton) allows the interaction between the Lamin nucleoskeleton and the cytoplasmic cytoskeleton (F-actin, microtubules, intermediate filaments) (Kirby & Lammerding,

2018) has been shown to be key in ensuring F-actin organization at the immune synapse as well as centrosome and lysosome polarization, both in B and CD4⁺ T lymphocytes (González-Granado et al., 2014; Obino et al., 2016; Ulloa et al., 2022).

The nucleus and the centrosome are very closely associated in lymphocytes, and the generation of the centrosome-nucleus polarity axis necessitates extensive cytoskeletal rearrangements and in particular F-actin-microtubules interactions. Indeed, the centrosome of lymphocytes is physically linked to the nucleus through nesprins by a centrosomal pool of F-actin, which is cleared in a proteasome-dependent manner upon lymphocyte receptor engagement to allow centrosome polarization and docking at the immune synapse (Bello-Gamboa et al., 2020; Cassioli et al., 2021; Ibañez-Vega, Del Valle Batalla, Saez, Soza, & Yuseff, 2019; Obino et al., 2016). Centrosome polarization has been shown to also rely on changes in microtubule dynamics, in particular increased microtubule stability, growth rate, and, finally, global cell tubulin increase (Andrés-Delgado et al., 2012; Filbert, Le Borgne, Lin, Heuser, & Shaw, 2012; Hooikaas et al., 2020; Inoue et al., 2019; Sáez et al., 2019). Many other mechanisms have been described to support centrosome polarization and docking at the immune synapse, most involving F-actin remodeling: actin clearing both at the centrosome and at the center of the immune synapse as well as F-actin polymerization at the synapse periphery (J. C. Wang et al., 2017). Conflicting results suggest that only F-actin clearing, but not polymerization, is important for centrosome polarization (Obino et al., 2016; Pineau et al., 2022; J. C. Wang et al., 2017). These disagreements are likely due to the challenge of distinguishing the role of F-actin in lymphocyte signaling and its role in controlling microtubule cytoskeletal rearrangements. Centrosome polarization has also been linked to the recruitment of dynein at the immune synapse by Par3 (Reversat et al., 2015), pulling it towards this region through microtubules, combined with myosin II pushing from the rear of the cell in CD4⁺ T lymphocytes (Liu, Kapoor, Chen, & Huse, 2013), which is likely not the case in B lymphocytes where myosin II is required at the immune synapse for mechanical antigen extraction (Kumari et al., 2019; J. C. Wang et al., 2022). Surprisingly, while CD4⁺ T cells, CD8⁺ T cells and B cells all tend to detach the centrosome from the nucleus, transport and dock it to the synaptic membrane, all these steps do not appear to be indispensable for

all immune synapse's functions. Indeed, while effector CD8⁺ T cells need to dock their centrosome to the immune synapse to succeed in target cell killing (Tsun et al., 2011), the centrosome does not need to be dissociated from the nucleus to accomplish this task (Lui-Roberts et al., 2012). Even more surprising, centrosome polarity has been described to be dispensable for polarized cytokine secretion at the immune synapse of effector CD4⁺ T cells, in a study pointing to a stronger role of polarized actin remodeling at the immune synapse in localized accumulation and secretion of cytokines (Chemin et al., 2012).

Organelles repositioning. Centrosome repositioning implies the reorientation of the microtubule network and allows the transport of many cell components (signaling molecules, Golgi apparatus, mitochondria, vesicles...) to the immune synapse. Many different types of vesicles needed for the different types of immune synapses (recycling endosomes, lysosomes, lytic granules, multivesicular bodies) have been described to be transported to the immune synapse on microtubules, in a dynein-dependent manner (Nath et al., 2016; Yuseff et al., 2011) (see Fig.2). Their different nature is directly linked with the differences in functions between the different types of lymphocytes, but they have all been shown to be important for supporting immune synapse function, mostly by fusing with the synaptic membrane to release their content, with this process relying on transport on microtubules, local actin remodeling (Ritter et al., 2015, 2017), and recruitment of fusion-related proteins to lysosomes (e.g. Vamp7 (Obino et al., 2017), Rab27 (J C Stinchcombe et al., 2001)) and the exocyst complex to the synaptic membrane (Sáez et al., 2019). In B cells, failure to secrete proteases at the immune synapse (when mechanical antigen extraction is not possible) has been shown to be greatly detrimental to antigen acquisition, and therefore to further presentation to CD4⁺ T cells and continuation of the immune response (Obino et al., 2017). Defects in lytic granules polarization and secretion in CD8⁺ T cells, found for example in Wiskott-Aldrich syndrome patients suffering from actin polymerization defects, or in patients suffering from defects in the adenomatous polyposis coli (APC) gene (familial adenomatous polyposis), leads to reduced efficiency of target cell killing, possibly to collateral damage in the case of non-polarized secretion, and can explain in part the higher

risk of infections or cancers in these pathologies (De Meester, Calvez, Valitutti, & Dupré, 2010; Juzans et al., 2020).

B cells the need to internalize antigen into endosomes, a process mostly described to occur through clathrin-coated pits (Roberts et al., 2020), then to process it in degradative compartments. Hence, B lymphocytes likely have additional specific vesicle trafficking and fusion requirements, for example for antigen processing (Hernández-Pérez et al., 2020; Vascotto et al., 2007).

Dynamical aspects. Immune cells must be able to polarize, depolarize and repolarize quickly to accomplish their function. The time of interaction must be tightly regulated: the contact must be strong (long) enough to provide sufficient activating signal but weak enough to be broken when needed. To understand the mechanisms that regulate this timing, it is crucial to accurately measure the kinetic of polarization.

Microfluidic assisted presentation assays have been recently used to study, by live microscopy, the kinetics of polarization of B cells to establish the sequence of events leading to polarization: signal and actin accumulation at the synapse (peaking within the first 2 minutes) are followed by centrosome rotation towards the synapse (5 minutes), Golgi re-orientation (7 minutes), and lysosome accumulation (10 minutes). The nucleus is reoriented together with the centrosome and is ultimately pushed towards the rear of the cell later (>15 minutes) (Pineau et al., 2022).

In CD8⁺ T cells, it has been recently shown that the rate of polarization depends on the strength of the TCR signal in a very subtle way: a stronger TCR signal, related to the affinity of the TCR for the antigen, results in higher chances of having long interaction time with the target cells, with more homogeneous and stronger Ca⁺⁺ response and more likely centrosome docking. Docking to the synapse is accompanied by degranulation, hence stronger signaling results in a more efficient killing. The pathways regulating the killing are conserved even at low affinities, but the rate of initiation of polarity depends on the signal of the receptor (Frazer, Gawden-Bone, Dieckmann, Asano, & Griffiths, 2021).

Novel approaches to study polarization kinetics. Immune cell polarity is classically studied by immunofluorescence microscopy. While several techniques have been developed to study the immune synapse formation from the molecular up to the cellular scale (TIRF microscopy, lipid bilayers, etc), many dynamical processes in polarity are still studied by fixation at different time points. Recently promising presentation tools and analysis methods adapted to study the dynamics of polarization at the immune synapse have been introduced. Measuring the dynamics of events occurring at the synapse and inside the cells on the scale of 15-30 minutes with a time resolution of at least a minute requires (i) control of the beginning of the interaction, (ii) a good spatio-temporal resolution compatible with reduced photodamage, and (iii) good statistics to account for cell-cell variability. These features make the observation more complicated than single time point kinetics. New imaging and cell manipulation approaches have become particularly effective in capturing 3D movies while specifically stimulating a single cell and accurately controlling the beginning of the interaction as well as the properties of the antigen-presenting object. For example, polarization can be studied following the encounter in microfluidic traps that allows multiplexed synchronized observations, compatible with high-resolution fluorescence (and confocal) microscopy (Dura et al., 2015; Mesdjian, Ruysen, Jullien, Allena, & Fattaccioli, 2021) (Jang et al., 2015) (Pinon et al., 2021). This method made it possible to measure the B cell polarization dynamics described above (Pineau et al., 2022).

Some findings described in the previous section for T cells (Ritter et al., 2015) have been possible thanks to microscopes particularly adapted for prolonged 4D acquisition, such as the Lattice Light Sheet Microscope which allows super-resolved volumetric images at a rate of about a frame every few seconds with low phototoxicity (Chen et al., 2014). Combinations of high-speed and high-resolution microscopy with microfabrication and microfluidics will improve the description of synapse formation and polarity acquisition in specific cases.

3.2 Forces and mechanics in polarity

Synapse formation, and the subsequent cell polarization, have been shown to be related to the mechanical properties of the substrate that presents the antigen. In this section, we will discuss the way mechanics influence polarity and how the cell can exert measurable forces related to synapse formation.

Both T and B cell receptor signals are stronger when antigen is presented on stiffer substrates (Wan et al., 2013) (Saitakis et al., 2017) (Blumenthal, Chandra, Avery, & Burkhardt, 2020). The link between signal and polarity is not clear, but there is evidence that more cells polarize their centrosome when stimulated with stronger ligands in the case of CD8⁺ T lymphocytes (Frazer et al., 2021). These cells are also more cytotoxic when the target cell is stiffer (Tello-Lafoz et al., 2021), an interesting property to exploit in potentiating checkpoint blockade immunotherapies, adding a mechanical component to immunosurveillance.

In several immune cell types, antigen recognition is followed by a global change in the mechanical (viscoelastic) properties of the cortex (Zak et al., 2021): the cortex becomes at the same time stiffer and more viscous in stimulated CD4⁺ T cells (Sawicka et al., 2017) (Jung, Zhou, Iden, Bischoff, & Qu, 2021), CD8⁺ T cells (Basu et al., 2016), B cells (Merino-Cortés et al., 2020), and neutrophils during phagocytosis (Zak et al., 2022). Most of these measurements were performed by presenting the antigen on a microbead and measuring the rheology of the cells with a microindenter. This is in accord with measurements showing that activated cells are indeed more rigid than naive ones (Bufi et al., 2015). Although these changes are not local, they are induced by a local stimulus and occur concomitant to cell polarization. Moreover, in some cases (CD4⁺ T cell) a local accumulation of actin at the synapse is visible as a stiff protrusive structure, called “punch” (Husson, Chemin, Bohineust, Hivroz, & Henry, 2011; Sawicka et al., 2017).

The formation of the immune synapse and the establishment of a polarity axis are accompanied by active deformations of the cortex and membrane that translate into measurable forces. When the

antigen is presented on a deformable substrate (such as gel surfaces or gel microbeads) the forces exerted by the cell on the substrate can be measured from the deformation of the substrate. These techniques (known as traction force microscopy) have allowed showing that CD8⁺ T cells use tensile forces generated at the periphery of the synapse to potentiate the action of perforin (Basu et al., 2016); integrins are concentrated at the synaptic side (at the periphery) to allow force application and secretion of cytotoxic granules in the center of the synapse in a mechanosensitive way (M. S. Wang et al., 2022). CD4⁺ T cells present a similar structure and force pattern (Bashour et al., 2014).

B cells exert contractile forces at the periphery and point-like protrusive forces at the center, these points corresponding to actin protrusions where the antigen is indeed internalized (Kumari et al., 2019). The mature synapse has a concentric pattern of actin (at the periphery), integrin (more to the center), and antigen-receptor cluster (at the center). One can speculate that this stereotypical shape, which is reflected by the force patterning and concentric actin flow, effectively maintains a niche where actin polymerization, endocytosis, and secretory machinery are concentrated. The maintenance of a single polarity axis and a single synapse allows the accumulation of resources at a single spot and exo-endocytosis of material occurs in isolation from the external environment.

4. Asymmetric cell division in lymphocytes

After activation at the immune synapse, mature naive T and B lymphocytes undergo many successive cell divisions to clonally expand and generate a large and diverse progeny, sometimes going through a process called *asymmetric cell division*.

Asymmetric cell division is a mode of cell division where cytoplasmic constituents are differentially inherited by the two daughter cells and is classically used to describe a mode of cell division giving rise to two daughter cells of different fates, in contrast with symmetric cell division where daughter cells are identical. This division mode has been mainly described in stem cells and confers them the ability to maintain a stem cell population while also generating differentiated cells, as asymmetric cell division can give rise to daughter cells of different fates. This process entails the establishment of a

polarity axis defining an asymmetric accumulation of inherited elements, along with the mitotic axis. This polarization process has classically been associated with the polarity proteins Scribble and Par3, each defining a pole (Pham, Sacirbegovic, & Russell, 2014), and the polarized accumulation of Numb, a Notch signaling inhibitor (Juergen A Knoblich, 2010; J A Knoblich, Jan, & Jan, 1995) (see Fig.3).

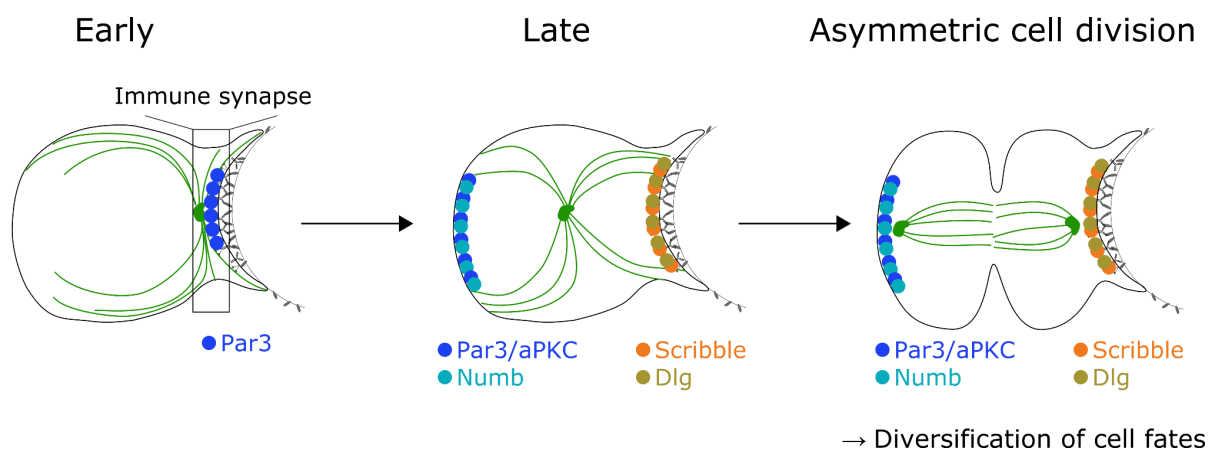
4.1 Asymmetric cell division in lymphocyte function

Mature lymphocytes have the specificity of not being terminally differentiated and are still able to divide many times upon activation and give rise to different progeny, for example, memory cells or effector cells. Some forms of asymmetric cell division have been observed in CD4⁺ T cells, CD8⁺ T cells, and B cells (naive and germinal center), and allow to obtain a robust and diverse population of lymphocytes (Barnett et al., 2012; Chang et al., 2007, 2011; Pham et al., 2014; Thauinat et al., 2012).

To generate immune memory while generating effector cells, naive T lymphocytes undergo asymmetric cell division following immune synapse formation. This process relies on the polarity axis defined by the immune synapse cell-cell junction, which defines the axis of asymmetric accumulation of fate determinants and the mitotic axis (Borsa et al., 2019; Chang et al., 2007, 2011; Oliaro et al., 2010). Although the polarity axis is conserved between the immune synapse and asymmetric cell division, polarity proteins reorganize as the immune synapse matures and the lymphocyte prepares for cell division: Scribble and Dlg-family proteins are retained in the proximal region, while aPKC/Par3 complexes are found in the distal part, opposite to their localization during immune synapse polarization, and together with Numb, a marker of asymmetric cell division regulating Notch signaling, and therefore cell fate (Chang et al., 2007; Oliaro et al., 2010). Asymmetric, polarized accumulation of fate determinants such as Numb (distal side, regulating Notch signaling), but also of surface receptors (e.g IFN γ receptors) or of proteasome content that can selectively degrade the fate determinant T-bet, will contribute to setting the fate of each daughter cell, likely together with other fate determinants not all formally identified to this day (Chang et al., 2007, 2011). Ultimately, naive T

cell activation by a membrane-bound antigen gives rise to effector T cells and memory T cells through asymmetric cell division, thus ensuring a robust T cell immunity through the diversification of cell populations (Buchholz et al., 2013). Preventing CD8⁺ T cell polarization for asymmetric cell division, for example through inhibition of aPKC-Par6 interactions and non-polarized segregation of Numb and aPKC, leads to decreased memory phenotypes of CD8⁺ T cells. Interestingly, memory CD8⁺ T cells can also undergo asymmetric cell division after rechallenge, and therefore maintain a memory pool while generating effector cells, similar to naive CD8⁺ T cells (Ciocca, Barnett, Burkhardt, Chang, & Reiner, 2012).

[Insert figure 3]: Figure 3: Asymmetric cell division in lymphocytes. In T cells, asymmetric cell division takes place at a late time after synapse formation, following the partition of Numb/Scribble and producing an effector and a memory cell.



Another, less canonical, form of asymmetric cell division has also been described in CD8⁺ T lymphocytes, where an asymmetric accumulation of lytic machinery leads, but not of fate determinants, results in two daughter cells with different killing capacities, but identical cell fates (Lafouresse et al., 2021).

In B lymphocytes, asymmetric cell division in naive cells has not been observed in terms of cell fate in the presence of soluble antigen (but has not been studied for B lymphocytes encountering

surface-tethered antigens and forming an immune synapse), and the asymmetric cell division regulator Scribble appears dispensable for mounting humoral immune responses (Duffy et al., 2012; E. D. Hawkins et al., 2013). However, (Thaunat et al., 2012) showed that naive B cells do polarize a very important cytoplasmic content during cell division, the internalized antigen, giving rise to two daughter cells of similar fate but different presentation capacities. Indeed, even after several rounds of B cell division, antigen-containing vesicles are mostly retained in one daughter cell, in a process that is believed to improve further presentation to CD4⁺ T cells, and therefore B/T cooperation and the generation of high-affinity antibodies. Asymmetric cell division leading to diversification of cell fate has however been described in germinal center B cells and relies heavily on cell-cell adhesion for the definition of a polarity axis for asymmetric segregation of molecules, and likely plays a similar role as in T cells and ensures the generation of memory and effector lymphocytes (Barnett et al., 2012).

4.2 Asymmetric cell division in immune cell development and aging

Addressing the role of asymmetric cell division in lymphocyte (and immune system) function in an organism is very challenging, as this process relies both on very widely-used polarity proteins -thus generating many effects at different levels of the organism- and on very redundant systems that develop numerous compensatory mechanisms (Pham et al., 2014). However, accumulating evidence highlights the importance of this polarity-based process in immune cell development, immune cell function, and the generation of immune memory.

Asymmetric cell division in immune cell development. Hematopoietic stem cells are the multipotent precursors giving rise to all blood cells and immune cells. They can undergo symmetric or asymmetric cell division to renew their population and produce new immune cell precursors throughout life, in a proportion regulated by their pro-renewal or pro-differentiation environment (Wu et al., 2007). Mature immune cells do not directly arise from hematopoietic stem cells, but rather from the generation and selection of many different precursors necessary to generate the diverse immune cell populations.

Asymmetric cell division has also been described during this process, termed immune cell development, and in particular during T cell development in the thymus. Indeed, T cell development relies heavily on Scribble-mediated asymmetric cell division of cells undergoing the β -selection stage (Pike, Kulkarni, & Pawson, 2011). During this step, precursors of T lymphocytes (DN3a) use spatial cues from their adhesion to stromal cells and interaction with the chemokine CXCR4 to establish a polarity axis and asymmetric segregation of Scribble, then Numb and α -adaplin, before dividing to obtain two daughter cells with different levels of these proteins and of specific fate determinants, leading to the renewal of DN3a-stage T cell precursors as well as the production of DN3b T cell precursors, one step further in T cell development (Pham et al., 2015; Pike et al., 2011). Loss of Scribble leads to defects in the asymmetric segregation of Numb and α -adaplin, defects in asymmetric cell division, and accumulation of DN3a T cell precursors (Pham et al., 2015; Pike et al., 2011).

Asymmetric cell division in aging. One of the hallmarks of aging of the immune system is the loss of ability to undergo asymmetric cell division by hematopoietic stem cells (Florian et al., 2018), as well as the decrease in asymmetric cell division in CD8⁺ T cells in general (Borsa et al., 2021). This decrease is mainly due to the repeated generation of short-lived/exhausted effector cells, and the decrease in asymmetric cell division abilities of naive CD8⁺ T cells. However, virtual memory CD8⁺ T cells, a specific category of CD8⁺ T cells that have never encountered an antigen but possess a memory cell phenotype (Haluszczak et al., 2009; Hussain & Quinn, 2019), retain the ability to do asymmetric cell division (Borsa et al., 2021). This leads to an accumulation of virtual memory CD8⁺ T cells during aging, which could compensate for the decreased proliferative capacities of naive CD8⁺ T cells and could be a mechanism for immune system resilience in aging, and maintenance of CD8⁺ T cell immune memory (Borsa et al., 2021).

5. Conclusions and perspectives

The three main occurrences of cell polarity in immune cell functions constitute an excellent example of the conservation of functional modules across cell types. The molecules we find maintaining polarity during migration are classically described in other migrating cells (Cdc42, Actomyosin regulators, etc), while the synapse has interesting parallels with developing embryo (Par3/6) and primary cilia of ciliated cells for intracellular transport (we refer the reader to the seminal paper (Finetti et al., 2009) or one of the many excellent reviews on the subject (Baldari & Rosenbaum, 2010; Douanne, Stinchcombe, & Griffiths, 2021)). Also, asymmetric cell division relies on Scribble/Numb partition as in stem cell differentiation. However, immune cell polarity has peculiarities that are of interest beyond immunological applications: immune cells quickly acquire a polarity axis but can lose it or change it within seconds. A rather unexplored subject is the link/competition between motility and immune synapse polarity/immune synapse formation and polarization, or more generally, the robustness of polarity. When a cell in a resting, unpolarized state acquires a polarity axis and migrates, it breaks its spherical symmetry. When it encounters another cell and forms an immune synapse, it has to re-polarize (by keeping a polarity axis or by depolarizing and repolarizing). When the synapse is mature and the function of the cell is accomplished (e.g. a CD8⁺ T cell has killed the target cell), another polarity axis needs to be acquired. For example, it has been shown that CD4⁺ T cells, when mildly stimulated, form a transitory synapse called kynapse, that might allow them to find more efficiently for cells presenting the antigen of highest affinity (Moreau et al., 2015). In CD8⁺ T cells, the centrosome is at the back (uropod) during migration and translocates to the synapse when the cell encounters a target. When the granules are secreted, the centrosome reverts to a position proximal to the nucleus before detaching and migrating to search for another target (Ritter et al., 2015). In all cases, the timing of these responses is finely regulated by the strength of receptor signaling, internal signaling cascades, and as seen recently, external mechanical cues. How the cell integrates this information is still an open subject of research.

Immune cells need to polarize long enough to gather signals, but not too long to be able to move to their next step. This observation leads to another related question: how can a cell maintain a given polarity axis? How can it disrupt it and reform it within minutes? Some clues come from mathematical modeling. A simplified model for polarity, proposed in the context of amoeba migration, is the Local Excitation/Global Inhibition (LEGI): local positive feedback and a global negative regulator of polarity is enough to explain the stability of a single polarity axis (Xiong, Huang, Iglesias, & Devreotes, 2010) (Zhang, Guo, & Wang, 2014). In B cells a similar mechanism has been proposed where a local positive reinforces of the synapse (microtubules, acetylated upon BCR signaling, release GEF-H1 and activate RhoA, hence actin polymerization and myosin II contractility) competes with a global inhibition by the action of de-acetylases (HDAC6). This might provide a mechanism to reinforce and maintain a single immune synapse (Pineau et al., 2022).

Robustness has profound implications, for example, in engineering and improving the killing capacity of CAR-T cells or the effectiveness of a stimulating antigen for a B cell. It can also explain to what extent the signal provided by a receptor implicated in motility (chemokine receptors) or cell-cell contact (TCR-BCR and co-receptors) can be perturbed by external cues. Future studies will provide answers to these questions and possibly suggest new roles for cell polarity in immunity.

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