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Polarity and lymphocyte fate determination

John T. Chang

Department of Medicine, University of California San Diego, La Jolla, CA, USA

Abstract

Polarity within lymphocytes has been recognized to regulate a variety of processes, including migration, signaling, and the execution of effector function. It has been recently proposed, however, that this polarized behavior may serve a different purpose in lymphocytes that have not yet encountered their foreign antigen—to coordinate asymmetric cell division. Asymmetric division is an evolutionarily conserved mechanism allowing a single cell to give rise to two distinct daughter cells from inception. In this review, recent findings in polarity and asymmetric division in lymphocytes are discussed.

Introduction

Generating diverse progeny from a limited number of progenitor cells is a central problem for multi-cellular organisms. These rare cells face the challenge of continually producing terminally differentiated cells while also preserving a self-renewing lineage. Like progenitor cells, lymphocytes in the mammalian immune system must also balance the conflicting demands of terminal differentiation with self-renewal.

Lymphocytes are a key component of the adaptive immune system. CD4⁺ T lymphocytes orchestrate defenses specific to distinct classes of microbial pathogens, while CD8⁺ T lymphocytes defend against intracellular pathogens by killing infected cells. B lymphocytes serve as a third arm of adaptive immunity, producing antibodies specific against microbes. Lymphocytes circulate continuously through the blood and peripheral lymphoid tissues, patrolling for evidence of microbial invasion. During an invasion, a naïve lymphocyte, so-called because it has not encountered its foreign antigen, must give rise to at least two distinct classes of cellular progeny. Terminally differentiated, short-lived “effector” cells provide acute host defense, while long-lived “memory” cells are responsible for providing recurrent immunity should the microbe be encountered again [1].

For both CD4⁺ and CD8⁺ T lymphocyte responses, naïve lymphocytes must give rise to effector and memory cell progeny. For CD4⁺ T cell responses, additional choices must be made among various effector lineage fates. Distinct effector subsets are specialized against specific classes of microbial pathogens: T helper 1 (Th1) for intracellular pathogens, Th2 for parasitic worms, and Th17 for fungi and extracellular bacteria [2–4]. A fourth effector lineage, T follicular helper cells (T_{FH}), has the unique ability to home to B cell follicles and induce antibody production by B lymphocytes [5]. In addition to these effector choices, naïve CD4⁺ T lymphocytes can also develop into inducible T regulatory cells (iTreg) that

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Corresponding author: Chang, John (changj@ucsd.edu).

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serve to limit the extent of inflammation caused by their effector siblings [6,7]. Thus, naïve CD4⁺ and CD8⁺ T lymphocytes give rise to diverse progeny during an immune response to microbial pathogen.

How is this cellular diversity achieved during an immune response? While our circulating lymphocytes are collectively capable of recognizing virtually any microbial invader, the price paid for this breadth of recognition is an extremely limited number of lymphocytes specific for any given microbe [8,9]. Compounding this problem is the enormous four-dimensional challenge of patrolling lymphoid tissues throughout the body, making it difficult to imagine how we even have one microbe-specific naïve cell in the right place at the right time. Given these considerations, how is diverse cellular progeny generated? One possibility is that naïve lymphocytes could be exclusively fated to become effector cells or memory cells, but not both (“one naïve cell, one fate”) [10,11]. A limitation to such a model, however, is that cellular diversity could not be achieved if the immune response were initiated by a single responding naïve lymphocyte. An alternative possibility is that a single naïve cell could give rise to both effector and memory cells (“one naïve cell, multiple fates”).

Several recent studies using complementary approaches have revealed that a single naïve lymphocyte is indeed capable of giving rise to progeny with more than one fate [12,13]. Busch and colleagues used a single cell adoptive transfer method to demonstrate that a single CD8⁺ T cell can give rise to effector and memory cells during a microbial challenge with the intracellular pathogen *Listeria monocytogenes* [13]. Schumacher and coworkers developed a novel barcoding approach to address the ontogeny of effector and memory CD8⁺ T cells [12]. Thymocytes were labeled with unique genetic tags (“barcodes”) and injected intrathymically into recipient mice to create a pool of naïve barcode-labeled T cells. This study demonstrated that effector and memory cells are derived from the same naïve T cells. Moreover, this finding of shared ancestry between effector and memory cells was true in T cells activated by either weak or strong signals, as well as in different infectious models across several anatomic sites. Together these elegant studies suggest that the ability of a single naïve cell to give rise to diverse cellular progeny may be a general feature of adaptive immune responses.

What remains unresolved, however, is the fundamental question of how a single naïve lymphocyte gives rise to effector- and memory-fated progeny. One possibility is that lymphocyte fate is determined by the cumulative effect of signals on responding naïve T cells and their progeny. In such a model, signals received by the progeny of the initial responding T cells are integrated to ultimately determine lymphocyte fate. Such signals can include additional antigenic stimulation or pro-inflammatory cytokines [14,15]. These studies have suggested a model in which all progeny of responding naïve T lymphocytes transit through an effector phase before acquiring characteristics of long-lived memory cells. In light of recent evidence suggesting that T lymphocyte fates diverge early during an immune response [16,17], however, could an alternative mechanism enable a single responding naïve cell to give rise to differentially fated daughter cells?

Polarity and asymmetric division as a regulator of CD8⁺ T lymphocyte fate

Balancing the demands of terminal differentiation with self-renewal is a problem that a variety of cell types in different tissues across multiple species have solved using a mechanism known as asymmetric division [18]. Asymmetric division is an evolutionarily conserved process that allows a single cell to give rise to two daughter cells that are differentially fated from their inception. Such a strategy allows a rare progenitor cell to give rise to a terminally differentiated lineage without depleting itself. While the specific details

differ, the fundamental principles underlying asymmetric division have been elucidated in studies using the *C. elegans* zygote, *Drosophila* neuroblast, and other model systems [19]. An axis of polarity is established during interphase, guided by an extrinsic polarity cue, such as contact with the niche in the case of stem cells. This axis of polarity is often established by members of an evolutionarily conserved network of polarity proteins, known as the partitioning defective (Par) proteins, which include the Par3-Par6-atypical PKC (aPKC) and Discs large (Dlg)-Scribble (Scrib)-Lethal giant larvae (Lgl) polarity complexes [20,21]. During mitosis, the axis of polarity allows the mitotic spindle to be properly oriented, permitting cell fate determinants to be asymmetrically localized [22]. This coordination of spindle orientation and asymmetric localization of determinants ensures their unequal inheritance by the daughter cells. A variety of determinants can be asymmetrically inherited in this fashion, including proteins, organelles, membrane components, and DNA [19].

It has been known for a number of years that engagement of a T lymphocyte by an antigen presenting cell is associated with polarization of signaling and adhesive components towards the site of contact, termed the immune synapse [23]. The formation of the immune synapse is accompanied by a substantial reorganization of several cellular components, including the microtubule-organizing center, the Golgi apparatus, and several members of the polarity network, including Par3, Par6, Scrib, and Dlg [24–27]. Many of the insights into the immune synapse have been gleaned from studies in effector T cells, where this polarized behavior is thought to coordinate the execution of effector function through polarized release of cytokines and cytolytic granules [28,29]. However, a body of recent evidence suggests a distinct purpose for the immune synapse in activated naïve lymphocytes—to reorganize cellular components in preparation for an asymmetric division.

Examination of a naïve CD8⁺ T lymphocyte responding to a microbial pathogen suggested that it exploits its contact with an antigen presenting cell as a provisional surface on which to organize polarity and subsequent asymmetric division [30]. Activated naïve T cells were found to asymmetrically segregate several classes of proteins during interphase, including immune receptors, signaling molecules, and polarity proteins including members of the aPKC-Par3-Par6 and Scrib-Dlg-Lgl complexes (Figure 1a). Phenotypic and functional analyses of the daughter cells following division suggested that the daughter cell “proximal” to the provisional contact with the antigen presenting cell undergoes terminal differentiation as an effector cell, whereas the daughter cell “distal” to the contact site eventually gives rise to a self-renewing memory cell [30]. These findings provided the first experimental evidence that T lymphocytes undergo asymmetric division during an immune response to a microbial pathogen.

Further evidence of asymmetric CD8⁺ T cell division was provided by Russell and colleagues in a subsequent study that used an *in vitro* cell culture system to model T cell activation [31]. One feature of this cell culture system was the ability to capture the interaction between T lymphocyte and antigen presenting cell. Although it has not yet been established *in vivo* whether or not a T cell divides while in contact with its antigen presenting cell, this cell culture system enabled the evaluation of mitotic spindle orientation, a critical step in coordinating asymmetric division [31]. Using an experimental approach that disrupted trimeric G protein signaling mediated by Dlg and Partner of Inscuteable (Pins), these investigators provided evidence supporting a role for this polarity complex in regulating mitotic spindle orientation and asymmetric division, at least *in vitro*. It remains to be formally demonstrated, however, whether disruption of mitotic spindle orientation results in alterations in the ability of an activated naïve T cell to give rise to diverse progeny during an immune challenge *in vivo*. Such evidence would support the hypothesis that asymmetric division indeed influences T lymphocyte fate during an immune response.

The possibility of asymmetric division in other lymphocytes

Can asymmetric division occur in lymphocytes other than CD8+ T cells? Indeed, CD4+ T lymphocytes responding to infection with *Leishmania major* have been shown to asymmetrically segregate certain proteins during their first division, including immune receptors and the receptor for the cytokine IFN γ [30]. How asymmetric CD4+ T cell division might give rise to the diversity of CD4+ T cell fates, which include regulatory, memory, and several effector lineages, however, remains unclear. Unlike naïve CD8+ T cells, which require only a single interaction with antigen presenting cells to undergo differentiation into effector cells [32], CD4+ T cells may require additional encounters with antigen presenting cells in order to differentiate [33]. This observation raises the possibility that activated naïve CD4+ T lymphocytes could undergo a series of sequential asymmetric divisions, giving rise to all possible cell fates, with the predominant cell type being dictated by the microenvironment. Such a possibility is suggested by the observation that even in microbial infections that drive a predominant Th1 effector response, Th2 cells can be detected at low frequencies [34].

Recent work from several laboratories have provided additional evidence of early heterogeneity in the CD4+ T cell response. Using a model of lymphocytic choriomeningitis virus, Crotty and colleagues identified two distinct populations of CD4+ T cells within the first 48 hours after infection [16]. These populations could be distinguished by surface expression of the alpha chain of the receptor for the cytokine IL-2 (IL-2R α). Moreover, cells expressing high levels of IL-2R α exhibited a gene expression profile characteristic of effector cells, whereas cells expressing intermediate levels of IL-2R α resembled T follicular helper cells. Using a model of *Listeria monocytogenes* infection, Jenkins and coworkers observed two distinct CD4+ T cell populations within the first 72 hours of infection with gene expression profiles characteristic of Th1 effector cells and memory cells [17]. These two populations were distinguishable by their expression of IL-2R α as well as the chemokine receptor CXCR5. In light of prior evidence that IL-2R α has been shown to be polarized to the immune synapse during activation of naïve CD4+ T cells [35], it is interesting to speculate that this early heterogeneity might be a result of asymmetric segregation of IL-2R α during division.

In addition to evidence showing asymmetric division in CD4+ and CD8+ T cells, work from several groups has suggested a functional role for polarity proteins in regulating thymocyte development. Pawson and colleagues demonstrated that depletion of Scribble in hematopoietic progenitors resulted in a partial block in thymocyte development [36]. Scribble-deficient progenitor cells failed to cluster appropriately and exhibited a defect in polarizing the adhesion receptor LFA-1 at sites of T cell-T cell interaction. As further evidence of a functional role of polarity in thymocyte development, Aguado and coworkers showed that numb, a modulator of Notch signaling, also appeared to play a role in thymocyte development, possibly by regulating asymmetric division of thymocytes [37].

Finally, several groups have recently demonstrated that B lymphocytes undergo asymmetric division [38–40]. Like cell-mediated immunity, humoral immunity requires diversity in cell fate, requiring the generation of antibody-secreting plasma cells and memory B cells. Using a peptide immunization model, Reiner and colleagues demonstrated that B cells in the germinal center, a specialized lymphoid structure in the spleen, underwent asymmetric division 8 days post-immunization [38]. These B cells asymmetrically segregated the receptor for the cytokine IL-21 (IL-21R) and the transcriptional activator Bcl-6, both of which regulate germinal B cell differentiation [38]. In addition, aPKC was asymmetrically segregated during division, being inherited preferentially to the side of the cell that received more IL-21R and Bcl-6 (Figure 1b). Batista and coworkers demonstrated that B cells can

asymmetrically segregate antigen that has been previously taken up from other antigen presenting cells [40]. These investigators showed that B cells accumulate antigen, maintain it in a polarized distribution for an extended time *in vivo*, then distribute it asymmetrically during division. Moreover, preferential inheritance of antigen was found to be correlated with an enhanced capability to present antigen. Together these results suggest that several classes of lymphocytes use asymmetric division as a strategy for generating cellular diversity or regulating lymphocyte function.

Mechanisms underlying asymmetric lymphocyte division and daughter cell fates

In other model systems of asymmetric division, unequal inheritance of fate-determining transcription factors by daughter cells allows them to acquire different fates from inception. In *Drosophila* neural stem cells, asymmetric segregation of the transcription factor Prospero enables it to act as a binary switch between terminal differentiation and self-renewal [41–43]. Another segregating fate determinant in *Drosophila* neuroblasts is numb, a repressor of Notch signaling [44]. Asymmetric segregation of numb during division yields unequal levels of Notch activity in the daughter cells, resulting in the establishment of distinct cell fates.

In asymmetrically dividing lymphocytes, at least two types of unequally inherited determinants could be envisioned to influence the fates of the daughter cells. Fate-determining transcription factors could themselves be asymmetrically segregated, thereby driving differences in cell fate directly. Indeed, several transcriptional regulators have been demonstrated to undergo asymmetric segregation in lymphocytes. T-bet, which drives effector differentiation in CD8⁺ T cells and the Th1 fate in CD4⁺ T cells, is unequally inherited during division of both types of T lymphocytes [45]. Furthermore, the transcriptional activator Bcl-6, which plays an important role in germinal center B cell differentiation, the T_{FH} fate in CD4⁺ T cells, and memory cell development in CD8⁺ T cells [46], also appears to undergo asymmetric segregation during B lymphocyte division, as discussed above [38]. It remains to be seen whether Bcl-6 and other transcriptional regulators known to regulate CD4⁺ T cell (GATA-3, ROR γ t, and Foxp3) and CD8⁺ T cell (Eomes, Blimp-1) fates are asymmetrically segregated during T lymphocyte division.

A second type of segregating determinant in lymphocytes could be receptors for various cytokines. The presence of certain cytokines and the absence of others in the microenvironment is a critical regulator of lymphocyte fate, through the induction and/or repression of fate-determining transcription factors. Asymmetric inheritance of a cytokine receptor by one daughter cell, for example, could result in preferential upregulation of a transcription factor relative to its sibling cell that received less receptor. In dividing T lymphocytes, it appears that both the IFN γ R and T-bet are preferentially inherited by the putative effector-fated daughter cell [30,45]. After division, enhanced IFN γ signaling has been proposed to result in increased T-bet mRNA induction [47] that could reinforce the pre-existing differences in T-bet protein levels between the daughter cells. In this way, asymmetric segregation of cytokine receptors and fate-determining transcription factors during division may be complementary mechanisms to promote fate differences in the daughter cells.

While polarized segregation of transcription factors has been described in other model systems of asymmetric division, the mechanism by which T-bet is believed to be rendered asymmetric has not been previously reported. In dividing T lymphocytes, it appears that the degradation machinery, the proteasome, undergoes asymmetric segregation beginning in metaphase [45]. Targeting of T-bet for proteasomal degradation, which is dependent on phosphorylation by the inducible T cell kinase ITK, also appears to occur during metaphase.

Together these events may allow T-bet to undergo preferential degradation on the side of the cell with more proteasome, yielding one daughter cell that inherits more T-bet and less proteasome, and the other that inherits less T-bet and more proteasome (Figure 2). Identification of this mechanism adds to the repertoire of degradation-related pathways underlying asymmetric division that have been characterized in other model systems. Such degradation-related pathways include asymmetric segregation of the ubiquitin ligase, or even the ubiquitinated proteins themselves [48,49]. Intriguingly, the observation that asymmetry of certain fate-determining transcription factors in *C. elegans* zygotes is dependent on proteasomal degradation [50] raises the possibility that the asymmetric proteasome segregation may be involved. These findings underscore the potential value of lymphocytes as a useful model system in which to uncover fundamental mechanisms underlying polarity and asymmetric division.

Polarity as a target of viruses

The hypothesis that the polarity network plays a critical role in regulating asymmetric lymphocyte division and immune responses is supported by the observation that microbial pathogens have evolved mechanisms to evade it [51]. It has been recognized that certain viruses encode proteins that target a domain, termed the PDZ domain, that is shared by many polarity proteins. PDZ domains facilitate protein-protein interactions, and are involved in a variety of cellular processes, including maintenance of intercellular junctions, polarity, and signal transduction [52]. While it has long been known that the human T lymphotropic virus type 1 (HTLV-1) encodes a PDZ-binding protein (PBM) called Tax, it is now clear that PBMs are encoded by viruses from several families, including hepatitis B virus, influenza, Dengue, tick-borne encephalitis, rabies, and Human Immunodeficiency Virus [51]. Although PDZ-binding proteins have a variety of targets and functional effects, a general principle is that these proteins tend to disrupt tight junction formation and the establishment of cellular polarity.

Tax, the PDZ-binding protein encoded by HTLV-1, is a transcriptional activator and is among the best studied PDZ-binding proteins, particularly in T lymphocytes. HTLV-1 is a delta-retrovirus that replicates in CD4+ T cells and causes leukemia and lymphoma in adults. Tax has been shown to bind to and mislocalize Scribble in human T cell lines [53,54], and also disrupts the ability of HTLV-1-infected T cells to polarize [55]. These observations raise the intriguing possibility that PDZ-binding proteins encoded by microbial pathogens might also disrupt asymmetric lymphocyte division, resulting in dysregulated immune responses.

Conclusions

Polarity has long been known to play a critical role in the regulation of lymphocyte signaling, migration, and execution of effector function. In recent years, it has been recognized that this polarized behavior may serve a distinct role in naïve lymphocytes recruited into an immune response—to coordinate asymmetric division. Many of our insights into how asymmetric division is regulated have been derived from studies in *C. elegans* and *Drosophila*, but the lymphocyte may now be poised to become a tenable model system in which to study asymmetric division. Asymmetric division has been observed in three major classes of lymphocytes, and several proteins not previously known to be asymmetrically segregated have recently been identified in lymphocytes. Nonetheless, several challenges lie ahead for the field.

One of the challenges will be to visualize polarized lymphocyte behavior and asymmetric lymphocyte division *in vivo*, as has been done in other models of asymmetric division.

Intravital imaging has been now been performed in virtually every organ, including the bone marrow, gut, liver, spleen, and brain [56–58]. In addition, it has become possible to achieve subcellular resolution *in vivo*, at least in migrating cells [59]. Moreover, technological advances allowing for ever-longer imaging experiments may soon enable visualization of subcellular structures within lymphocytes undergoing division. Combining these imaging advances with studies using strains of fluorescent fusion protein reporter mice should enable investigators to conduct fate-mapping studies *in vivo*.

An equally important challenge for the field will be to provide additional evidence that asymmetric division regulates lymphocyte fate and function. While it has been shown that several classes of determinants are asymmetrically segregated by different types of lymphocytes during division, there is less evidence supporting a functional role for this asymmetry. In this regard, characterization of the immune response in strains of mice genetically deficient or “knocked down” in various regulators of asymmetric division should be informative. Intriguingly, several such studies have suggested alterations in lymphocyte fate and function [60–62]. In addition, careful analyses of the early heterogeneity observed in CD4⁺ and CD8⁺ T lymphocyte immune responses may reveal whether this heterogeneity reflects differentially fated progeny arising from asymmetric division *in vivo*. The next few years are likely to yield exciting mechanistic insights into asymmetric lymphocyte division and its role in generating cellular diversity during immune responses.

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Figure 1.

Model depicting asymmetric division in T and B lymphocytes. **(a)** Prolonged interaction between a T lymphocyte and antigen presenting cell (APC) may allow the T cell to orient its plane of division and segregate asymmetric determinants relative to the plane of division. Certain signaling and fate determinants are inherited unequally by the synapse-proximal ("Proximal") and synapse-distal ("Distal") daughter cells, yielding distinct fates for the daughter cells. **(b)** Contact between a germinal center B lymphocyte and a follicular helper T cell (T_{FH}) may similarly allow the B cell to undergo asymmetric division.

Figure 2.

Model illustrating the mechanism by which T-bet may be asymmetrically inherited during T lymphocyte division. During interphase and prophase, T-bet (green ovals) is localized in the nucleus. After nuclear envelope breakdown, T-bet is displaced from chromatin and fills the volume of the cell. During early metaphase, the proteasome (blue squares) is reorganized asymmetrically towards one side of the cell. T-bet undergoes localized proteasome-dependent degradation on the proteasome-enriched side of the cell, with the remaining T-bet on the opposite side of the cell. During anaphase, T-bet becomes preferentially inherited by the daughter cell that inherits less proteasome. After division, the proximal daughter inheriting more T-bet gives rise to the terminally differentiated effector cell, whereas the distal daughter gives rise to the self-renewing memory cell.