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# Polarity and asymmetric cell division in the control of lymphocyte fate decisions and function

Mohammed Yassin[1,2,3] and Sarah M Russell[1,2,3,4]

Polarity is important in several lymphocyte processes including lymphocyte migration, formation of the immunological synapse, and asymmetric cell division (ACD). While lymphocyte migration and immunological synapse formation are relatively well understood, the role of lymphocyte ACD is less clear. Recent advances in measuring polarity enable more robust analyses of asymmetric cell division. Use of these new methods has produced crucial quantification of ACD at precise phases of lymphocyte development and activation. These developments are leading to a better understanding of the drivers of fate choice during lymphocyte activation and provide a context within which to explain the effects of ACD.

**Addresses**
[1] Immune Signalling Laboratory, Peter MacCallum Cancer Centre, East Melbourne, Australia
[2] Centre for Micro-Photonics, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, Australia
[3] Department of Pathology, The University of Melbourne, Australia
[4] Sir Peter MacCallum Department of Oncology, The University of Melbourne, Australia

Corresponding author: Russell, Sarah M (sarah.russell@petermac.org)

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## Introduction

Historically, lymphocyte polarity has been considered primarily as front-to-back polarity during lymphocyte migration and immunological synapse (IS) formation during antigen presentation. Both the role and mechanisms of cell polarity for these two processes are well established, and recent efforts have mostly been to determine the mechanistic details by which intracellular trafficking participates in the fine-tuning of the immune response [1]. In the last decade, a third major form of polarity, asymmetric cell division (ACD), has also been mooted, with much discussion of its role in lymphocyte development and activation [2]. In contrast to lymphocyte migration and IS formation, there is still no clear consensus as to when, where, how and why ACD might regulate lymphocyte development and activation. In recent years, however, major progress has been made in elucidating, the physiological effects of ACD in lymphocytes. This review describes new findings on the relationship between lymphocyte polarity and the immunological synapse, but focusses primarily on the substantial recent progress in research on ACD. These findings begin to shed light on the role of polarity and ACD in several aspects of lymphocyte development and function. We discuss the impact of these findings on the evolving view of the physiological role of ACD. We describe the emerging picture of the molecular and cellular activities that influence, and are influenced by, polarity and ACD in lymphocytes.

## Polarity and the IS

Several studies described below have recently reinforced the notion of causal links between cell polarity and the IS. These findings have also introduced new players to this functional connection. Cell polarity in solid tissues is frequently controlled by mutual antagonism between Scribble (SCRIB) and Par3 (PARD3) complexes. It is now well-established that during lymphocyte migration and IS formation, polarity proteins can both be polarized and regulate polarity. The most extensive data in lymphocytes is for the Par3 complex in B cells and the Scribble complex in T cells [2–7]. New data further implicates the Scribble complex member, Dlg1 (Discs large 1, DLG1), in T cell receptor signaling [8,9]. It has been recently shown that the Par3 complex is important in the B cell IS [3]. Hedgehog signaling, and associated vesicular trafficking regulators, has long been linked to the cell polarity phenomenon of primary cilia formation. Recent work has indicated that these proteins are also important in T cell IS formation [10,11,12•]. Downstream of T cell receptor signaling in the immunological synapse, Zap70 (ZAP70) controls the final stages of cytotoxic T cell polarity, recruiting the centrosome to the immunological synapse and polarizing cytotoxic granules [13]. The second messenger, diacylglycerol, at the IS also controls polarity and recruitment of the microtubule organizing center [14]. A new function that exploits the polarity established with the IS has been identified by Michael Dustin and colleagues, who have shown that vesicles enriched in T-cell receptors are secreted from the IS [15]. Together, these studies promote the idea that the IS is more than a concentration of signaling molecules. Rather, it is a structure that is dynamically integrated with other forms of cell polarity to orchestrate a broad range of cell behaviors.

## When in lymphocyte development and activation does ACD occur?

Asymmetric cell fate in non-lymphocyte systems can be controlled by asymmetry of a molecular fate determinant, asymmetry of organelles or size, and asymmetry in position (one daughter exposed to a cue and the other not). Most publications to date regarding lymphocyte ACD have focused on molecular asymmetry. ACD was first reported in hematopoietic stem cells and $CD8^+$ T cells in 2007 [16,17], More recently, ACD was observed in $CD4^+$ T cells [18], B cells [19,20] and DN3a thymocytes during the β-selection checkpoint [21•]. Additionally, the methods by which ACD is measured are becoming more sophisticated, and some of the early problems in interpretation have been revealed.

Because of the difficulty in monitoring asymmetry of cell divisions *in situ*, studies of ACD have involved *in vitro* models of lymphocyte development and activation or *ex vivo* analysis of dividing lymphocytes. One issue with *ex vivo* analysis is the use of the actin inhibitor, cytochalasin B to block cellular cytokinesis. It was recently shown that this treatment causes an increase in the polarization measured during ACD [22]. The efficiency of cell extraction from tissues might also influence experimental results. For instance, it was demonstrated that inefficient extraction of cells from lymph nodes might result in biased populations including migratory lymphocytes, rather than lymphocytes engaged with dendritic cells [23,24]. These issues, and recent studies showing that hematopoietic reconstitution using injected hematopoietic stem cells can alter the biology of lymphocytes [25], highlight that neither *in vitro*, *in vivo*, or *ex vivo* analyses are sufficient by themselves. Instead, a combination of these approaches is required to define the context in which ACD occurs.

Moreover, we do not know what degree of asymmetry of any molecule is sufficient to drive asymmetric cell fate. Thus, consequences of measured asymmetry are difficult to predict. Early measures of asymmetry were based on subjective scoring or simple ratios of the relative detection of proteins using fluorescent markers. However, new methods allow for automated, high-throughput and controlled estimations of the degree of polarization [21•,26–28]. This improved quantification, and recognition that the degree of polarity (rather than an arbitrary decree of asymmetric or symmetric) should be assessed, will allow for more sophisticated modeling and experimental testing of the role of ACD in lymphocyte fate determination.

## What aspects of development and activation do polarity and asymmetric cell division influence in lymphocytes?

A role for ACD in hematopoietic stem cells was readily accepted by the field, likely because ACD is known to influence self-renewal of stem cells in many model organisms, and subsequent research focused more on mechanistic and functional aspects of ACD in hematopoietic stem cells (detailed below). Interestingly, recent findings have suggested that stem cell homeostasis in mammals may depend less on ACD and more on a process of clonal drift, whereby symmetric amplification of stem cells from one clone can fill any gaps left by deletion of other clones (reviewed in [29,30]). How this reflects on the role for ACD in blood homeostasis has not been explored.

In contrast to hematopoietic stem cells, there has been a more heated debate over ACD's relative importance in determination of fate of mature lymphocytes. Remarkably, far more publications have promoted differing beliefs as to whether ACD plays no role, or a deterministic role, than have assessed the mechanisms and molecular or cellular consequences of ACD in T cells (see for instance [31,32]). This trend, however, has abated in the last 2–3 years. Several exciting new approaches to assessing the different intrinsic and extrinsic parameters that can influence T cell fate at different stages of the immune response (notably related to the choice between effector and memory differentiation) have led to a clearer view of the likely role for T cell ACD [33]. These findings, and some recent breakthroughs in assessing the role of different polarity regulators in the T cell response, have set the stage for more fact-based prediction of the precise physiological role of ACD.

Many arguments against a role for ACD in T cell responses are in fact against an absolute (singular) deterministic role, but do not exclude a contribution of ACD to T cell responses (e.g. [34–37]). A range of possible effects of ACD in T cell response are suggested from findings in model organisms and solid tissues. The influence of ACD on the progeny of most cell types can be broadly divided into self-renewal or bifurcation of cell fate (Figure 1). Two seminal studies showed definitively that T cell responses do not follow the type of stereotypical pedigree compatible with an absolute role for ACD in dictating bifurcation of fate at the first division, but rather that each founder naïve T cell yielded a remarkably diverse range of progeny [34,38]. Despite this finding and the clear precedent for a subtle influence of ACD in many cell systems, a consensus on a role for ACD in T cells has been impeded by debates that consider only the most extreme scenarios. Thus, the argument has frequently been whether or not ACD would dictate that one daughter yields memory cells and the other effector cells. A more constructive discussion would consider whether ACD works with other factors to influence fate. Similarly for B cells, it is not clear what bifurcation in fate might be influenced by ACD. Two recent studies showed less than 20% asymmetry in the proliferation and differentiation rates of progeny from B cell siblings [22,39]. This suggests that any divergence is unlikely to be universally adopted by all B cells, which is compatible with the fact that