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Cell polarisation and the immunological synapse

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Directed secretion by immune cells requires formation of the immunological synapse at the site of cell-cell contact, concomitant with a dramatic induction of cell polarity. Recent findings provide us with insights into the various steps that are required for these processes: for example, the first identification of a protein at the centrosome that regulates its relocation to the plasma membrane; the use of super-resolution imaging techniques to reveal a residual actin network at the immunological synapse that may permit secretory granule exocytosis; and the drawing of parallels between primary cilia and IS architecture. Here we discuss these and other novel findings that have advanced our understanding of the complex process of immunological synapse formation and subsequent induced cell polarity in immune cells.

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Introduction

The rearrangement of cell components to form the distinctive immunological synapse (IS), illustrated in Fig. 1, occurs when immune cells polarise in response to recognition of an antigen presenting cell (APC) [1,2]. Receptors involved in APC recognition and intracellular organelles both polarise towards the IS, permitting the transmission of signals and precise secretion towards the APC. IS formation and induction of cell polarity are especially important for cytotoxic T cells (CTL) and natural killer (NK) cells as these events allow the cells to use polarised secretion to destroy APCs, the stages of which are depicted in Fig. 2. Although the IS and cell polarisation in immune cells are tightly linked, we still do not completely understand this interplay, and much of the current research is focused on extending our knowledge in this area. This review will focus on the secretory synapses formed by CTL and NK cells.

Insights from new techniques

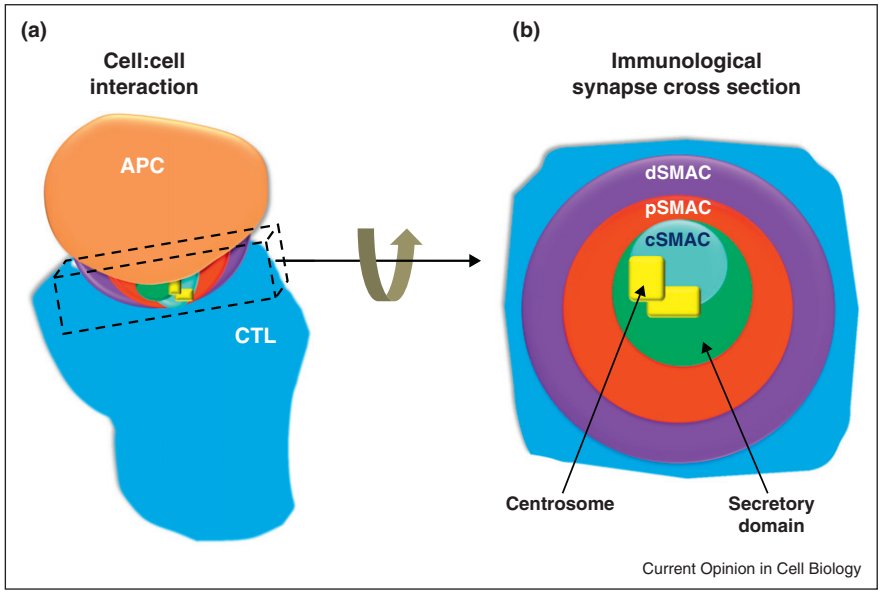
The dynamic formation of the IS has been uncovered in greater detail recently due to increased use of advanced microscopy techniques. A number of different approaches have been employed to obtain high-resolution images of the cell–cell interface, yielding new insights into the formation of the IS. Those of interest are summarised in Fig. 3 and discussed subsequently.

Optical tweezers can rotate interacting cells so that the interface lies in the higher resolution *xy* plane instead of the *xz* plane (Fig. 3a). This method avoids combining *xy* planes, which generates a low-resolution image in the *z* plane. Its use is nicely demonstrated in an in-depth assessment of interactions of signalling components SLP-76 and LAT, adaptor proteins that are phosphorylated downstream of the TCR thus allowing signal propagation. The study suggests that it is vesicular LAT that is important for signal transduction [3••,4]. Using a similar principle, conjugate orientation in micropit arrays also allows positioning of the interface in the horizontal imaging plane [5] (Fig. 3b).

Total internal reflection fluorescence microscopy (TIRFM) on cells adhered to planar lipid bilayers also generates high-resolution images of the IS (Fig. 3c) and has been used extensively to identify the mechanisms controlling the clustering of receptors into the central supramolecular activation complex (cSMAC, Fig. 1b). These assays have revealed receptor microclusters form at the periphery of the IS and migrate inward centripetally, controlled by F-actin flow [6]. Further microcluster TIRFM studies, complemented by super-resolution stimulated emission depletion microscopy (STEDM), revealed dynein-dependent movement along microtubules closer to the IS centre [7•], suggesting two distinct phases of movement as shown for B cells [8]. Photoactivatable linkages between T cell receptor (TCR) and peptide-major histocompatibility receptor (pMHC) monomers additionally demonstrated that TCR bound to pMHC are selectively recruited to the cSMAC [9].

Confocal imaging indicates that actin is cleared to the dSMAC of the IS [10], but two simultaneously published papers highlighted the benefits of super-resolution microscopy by revealing a residual actin network across the NK IS where secretion occurs [11•,12•] (Fig. 3d). This is more noticeable in the higher resolution STEDM images [12•] than the 3D-structured illumination microscopy [11•]. The authors suggest a role for the residual actin in granule secretion, discussed in 'Control of granule delivery'.

Figure 1



The immunological synapse (IS) in cytotoxic T cells (CTL).
The IS forms at the site of cell contact between CTL and APC (a), with a series of supramolecular activation clusters (SMAC) forming as receptors segregate into a characteristic bullseye pattern when viewed *en face* (b) the central SMAC (cSMAC) with clustered T cell receptors (TCRs) involved in target recognition; the peripheral SMAC (pSMAC) with integrins involved in adhesion and the distal SMAC (dSMAC) with excluded phosphatases (CD45) and actin. Polarised secretion from CTL is directed by the centrosome (see Fig. 2) [13], which contacts the cSMAC forming the secretory domain around this point [49].

Control of centrosome polarisation

A key event in IS induced cell polarisation is movement of the centrosome right up to the membrane at the edge of the cSMAC, initially observed in CTL [13] and more recently in CD4 [14••] as well as NK and NKT cells [15•] (Figs. 1 and 2). As the centrosome is the microtubule organising centre of CTL, its movement induces reorganisation of the intracellular microtubule cytoskeleton, which is thought to allow polarised secretion of lytic granules at the IS (see 'Control of granule delivery' and Fig. 2). Interestingly, although movement of the centrosome and its membrane docking were observed many years ago, the literature is yet to comprehensively explain how this precise and very unusual movement is controlled.

Many studies suggest that the cytoskeleton, along with motor proteins, may permit the centrosome to move to the IS. Dynein and related molecules have long been implicated in this process (Fig. 2v). Huse linked diacylglycerol (DAG) accumulation at the IS with dynein recruitment and centrosome polarisation [16], and more recently depletion of DAG kinase ζ revealed its participation in restricting DAG to the IS, although this study did not assess the subsequent effect on centrosome reorientation [17]. Proteins linking the cytoskeleton and membrane also appear to have a role; ezrin localises to the Jurkat IS along with the epithelial cell polarity protein Discs-large homolog 1 (Dlg1) [18]. This work also suggested ezrin regulates Dlg1 because ezrin depletion caused a modest decrease in Dlg1 IS localisation, with depletion of Dlg1 itself having some negative impact on the ability of the centrosome to polarise to the IS. Additionally, expression of key leucine–aspartate domains of paxillin, (a centrosome localised cytoskeletal adaptor protein best known for regulation of focal adhesions [19]) reduced CTL centrosome polarisation by 48% [20], again suggesting that cytoskeletal rearrangements have a role in induced centrosome movement.

Another intriguing candidate is casein kinase I-δ (CKIδ) which, when depleted from Jurkat cells, caused a strong reduction in centrosome polarisation to the IS [21••]. What is particularly fascinating about CKIδ is that it is one of the first centrosomal proteins found to influence centrosome positioning towards the IS, although CKIδ regulates microtubule growth and so may well control centrosome positioning in this way. CKIδ interacts with microtubule binding protein EB1 and the $p150^{glued}$ subunit of dynactin, and, since CKIδ localised to the centrosome but was not seen at the IS, these interactions seem more probably to be involved in microtubule anchoring. IQGAP1, which binds microtubules' plus-ends and links them to the actin cytoskeleton, has been shown to localise with actin in the dSMAC of the IS (Fig. 1) [13]. Interestingly, IQGAP1 knockdown studies in an NK cell line