The cytotoxic T-lymphocyte (CTL) immune synapse at a glance

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Abstract

The immune synapse provides an important structure for communication between immune cells. Studies on immune synapses formed by cytotoxic T lymphocytes (CTL) highlight the dynamic changes and specialised mechanisms required to facilitate focal signalling and polarised secretion in immune cells. In this Cell Science at a glance and the accompanying poster, we illustrate the different steps that reveal the specialised mechanisms used to focus secretion at the CTL immune synapse and allow CTL to be such efficient and precise serial killers.

Introduction

In its endeavour to fend off infection and cancerous growth, the mammalian immune system relies on both efficient communications between its cellular players as well as the ability to eliminate harmful agents in a precisely focused manner.

Immune cells can communicate directly with each other by forming close cell-cell contacts that have become known as immune synapses. In addition to this internal communication, the immune system makes use of the synapse during direct attack on infected and cancerous cells: the formation of immune synapses allows killer cells to address the challenge of specifically eliminating ‘dangerous’ cells whilst leaving healthy cells unaffected. Thus it is only after the establishment of the focused synapse interface that cytotoxic T-lymphocytes (CTLs) and natural killer (NK) cells deliver a cocktail of cytotoxic substances from specialised secretory lysosomes (cytolytic granules) to destroy the target. In this review we will provide an “at a glance” view of the CTL synapse highlighting features of its structure and roles in signalling, secretion and immunodeficiencies.

Structural features of the synapse

The formation of immune synapses involves the reorganisation of receptors involved in recognition and adhesion to form specialised functional domains at the interface between two cells. The first clear demonstration of distinct structural molecular patterning in response to target engagement came from work by Kupfer in $CD4^+$ T cells (Monks et al., 1998), with the rapid dynamics revealed using artificial bilayers (Grakoui et al., 1999). This showed T-cell receptor (TCR) clustering to the centre of the immune synapse or central supramolecular activation cluster (cSMAC) with protein kinase C (PKC)-θ and Lck surrounded by a ring of adhesion molecules, lymphocyte function-associated antigen 1 (LFA-1) and its adaptor talin, together referred to as the peripheral-SMAC (pSMAC). Subsequent immunofluorescence imaging has revealed an accumulation of actin surrounding the pSMAC, sometimes referred to as the distal-SMAC (dSMAC), creating a now well-known 'bulls-eye' configuration (see poster).

A similar structure was subsequently identified in CD8 CTLs with a discrete secretory domain next to the cSMAC and within the pSMAC (Potter et al., 2001; Stinchcombe et al., 2001b). These organised synapses between cells are widely adopted throughout the immune system, with similar layouts being used for both B and T-cell activation and even phagocytosis of particulates by macrophages (Freeman et al., 2016; Goodridge et al., 2011; Niedergang et al., 2016).

Another important feature of the synapse structure is the reorganisation of the cytoskeleton. The microtubule organising centre (MTOC) has long been known to polarise towards the synapse in $CD4^+$, $CD8^+$ and NK cells (Geiger et al., 1982; Kupfer and Dennert, 1984; Kupfer et al., 1983; Kupfer et al., 1985), and more recently it has been shown that one of the centrioles at the centre of the MTOC contacts the cell membrane next to the cSMAC (see Box 1), focusing secretion next to the point of TCR signalling (Stinchcombe et al., 2001b). This ensures precise secretion of the cytotoxic components perforin and granzymes and may also focus the delivery of the alternative cell death mediator FasL (Bossi and Griffiths, 1999; Kagi et al., 1994; Stinchcombe et al., 2006; Tschopp and Nabholz, 1990).

TCR signalling

CTLs identify their target cells through TCRs, whose signalling drives the dramatic reorganisation of the CTL cytoskeleton that goes with the establishment of a synapse.

Over the last 20 years, the biochemical outline of TCR signalling (see Box1) has been extended with improving microscopy techniques and the importance of the spatio-temporal dynamics of the process is now widely appreciated. Active TCR signalling is associated with the movement of small groups of TCR and LAT molecules (microclusters) from the synapse periphery toward the c-SMAC, where TCRs are endocytosed to a recycling endosome. Once internalised, TCRs may be redelivered to the synapse or selectively trafficked for degradation, and so either enhance or diminish signalling (see poster). Along with direct players in the TCR cascade, inhibitory molecules and cytokines may also be delivered through vesicles to the synapse, both modulating signalling and communicating with the antigen-presenting cell (APC) independently of cytolytic granules (Purbhoo, 2013; Soares et al., 2013).

One of the pathways that most recently has been implicated in TCR signalling is the Hedgehog (Hh) pathway (see poster). Hh signalling is the trademark of signalling in the primary cilium, a structure absent only from haematopoetic cells, and with surprising structural similarities to the synapse (Wheatley, 1995). In $CD8^+$ T cells, Hh signalling is initiated by TCR signalling and causes the intracellular activation of Patched 1 or 2 (Ptch1/2) by Indian hedgehog (Ihh) on vesicles within the T cell. This inhibits repression of Smoothened (Smo) by Ptch1/2, thereby activating Gli1 and driving expression of Hh target genes. In $CD8^+$ T cells, a key Hh target gene encodes the protein Rac1, which plays a critical role both in actin reorganisation and centrosome polarisation to the synapse. Inhibition of Smo, either genetically or by use of chemical inhibitors, disrupts CTL-mediated killing (de la Roche et al., 2013).

Recently, the importance of mechanical force in formation of the synapse has become a subject of increasing investigation. To attach and kill a target cell, the CTL must latch on tightly to its target and this requires the activation of the integrin LFA-1 (Hogg et al., 2011). Work from the Burkhardt lab in $CD4^+$ T cells has shown that achieving fully activated LFA-1 requires F-actin flow, with intercellular adhesion molecule 1 (ICAM-1) on the APC side of the synapse providing physical resistance to promote this effect during synapse formation (Comrie et al., 2015a; Comrie et al., 2015b). Interestingly, progress in measuring 2D binding kinetics has revealed similar roles for force in promoting adhesion with catch-bonds being formed by P-selectin, an adhesive molecule involved in CTL recruitment to sites of inflammation (Hirata et al., 2002; Marshall et al., 2003). Such catch

bonds have recently been implicated in the ability of TCRs to distinguish between agonist and altered peptide ligands, which whilst they remain stimulatory, result in greatly reduced killing efficiency; however, the underlying biology has yet to be fully explored (Liu et al., 2014).

CTL immune synapse formation: sequence of events

The development and refinement of high-speed live-cell imaging techniques has fuelled the investigation of the dynamics of synapse formation and CTL-mediated killing. Over the course of the past 10 years, the order and timing of some key steps in the attack have been unravelled, although some of the details vary somewhat depending on the technique and cell system used.

In vitro, when placed on a glass surface, CTLs migrate with a lamellipodium at the front and a uropod at the rear (see poster). As soon as a target cell is recognized, CTLs stop migrating and accumulate F-actin at the contact site. This is followed by a reduction in F-actin at the centre of the contact site within one minute after initial contact (Ritter et al., 2015). As a consequence, an F-actin ring appears at the edge of the interface which in the dSMAC. At the same time, TCR microclusters gather at the centre of the interface to form the cSMAC.

During CTL migration the centrosome (MTOC) is located away from the leading edge, behind the nucleus, in the uropod. When a target encounter triggers TCR signalling, the centrosome starts moving towards the immune synapse (Kuhn and Poenie, 2002). It is thought that ‘pioneer’ microtubules link the centrosome to the synapse interface and their shortening and the motor protein dynein act together to reel the centrosome to the synapse (Combs et al., 2006; Yi et al., 2013). The centrosome finally docks at the plasma membrane by the cSMAC, in a region where F-actin is depleted. It takes about six minutes from the cell-cell contact to centrosome docking at the synapse (Ritter et al., 2015) (see poster).

As cytolytic granules cluster around the centrosome, they move together with the centrosome towards the synapse where they release perforin and granzymes into the space between the CTL and the target (Ritter et al., 2015). Following the release of granule contents, perforin facilitates transport of granzymes into the target, which trigger rapid target cell death. Finally, the CTL detaches from the dying target cell and moves on to find the next target. A new lamellipodium is formed distant from the immune synapse.

The centrosome detaches from the synapse membrane and a new uropod is formed as the CTL moves away (Ritter et al., 2015). Intriguingly, the signal to detach appears to be dependent upon the target cell's demise through caspase activity (Jenkins et al., 2015).

The role of CTL centrosome polarisation in killing and similarities with cilia

Precise targeting of cytolytic granules towards exocytic sites opposite the target is mediated by an unusual mechanism that involves centrosome polarisation to the immune synapse membrane. On CTL activation, the centrosome moves from the back of the cell around the nucleus and docks with the plasma membrane within the immune synapse, at the boundary between the cSMAC and secretory domain (Ritter et al., 2015; Stinchcombe et al., 2006; Yi et al., 2013).

Centrosome polarisation is unusual, but also occurs in cells with cilia and flagella; here, the centrosome docks with the plasma membrane through the distal appendages of the mother centriole before extending to form a cilium or flagellum (Azimzadeh and Bornens, 2007). Intriguingly, centrosome docking at the CTL synapse appears to be remarkably similar to ciliogenesis, although a cilium does not form (Stinchcombe et al., 2015) (see Box 1).

Centrosomes are comprised of an older, more mature, 'mother' centriole characterised by two rings of appendages at the distal end of the centriole, and a younger 'daughter' centriole, which is derived from the mother during centriole replication and lacks appendages. The distal-most appendages are involved in membrane association, whereas the sub-distal are involved in microtubule organisation. CTL centrosomes dock at the immune synapse during target killing with the mother attaching to the membrane via the distal appendages of the mother centriole (Stinchcombe et al., 2015) (see poster). This organisation aligns the sub-distal appendages and associated microtubules under the plasma membrane at the secretory domain where granule contents are released. The mechanisms of centrosome docking at the CTL synapse and during cilia formation are also similar, with both processes requiring Cep83 (Stinchcombe et al., 2015; Tanos et al., 2013). However, once the centrosome has docked, the pathways diverge. In ciliated cells, the centrosome-end-regulating proteins CP110 and Cep97 are lost and cilia formation proceeds, whereas CTL mother centrioles retain the CP110-Cep97 complex on docking during killing and show no signs of cilia formation (Stinchcombe et al., 2015). Since it was recently shown that lymphocytes have the capacity to form cilia if CP110 is

depleted (Prosser and Morrison, 2015), it is likely that mechanisms involved in CP110 retention act to prevent cilia formation at the CTL synapse during killing (see poster). Preventing cilia formation, prevents stabilisation of CTL centrosomes and ensures centrosome docking is only transient, thereby allowing the multiple polarisation events that are required for sequential killing of several targets.

Other similarities between cilia and the immune synapse have also been found in $CD4^+$ T-cells, including a role for intraflagellar transport (IFT) proteins, which are required for cilia formation, in TCR recycling (Finetti et al., 2009; Finetti et al., 2014; Vivar et al., 2016). EM tomography reveals a very similar organisation of the centrosome and secretory compartments in $CD4^+$ T cells (Ueda et al., 2011), although centrosome docking is yet to be studied in $CD4^+$ T cells.

Targeted granule secretion at the immune synapse

The release of granule contents at the synapse is tightly controlled by a sophisticated protein machinery that coordinates the delivery, docking and fusion of granules at the plasma membrane (see poster). Malfunctioning of this machinery due to genetic defects in its components leads to the devastating immune deficiency condition familial haemophagocytic lymphohistiocytosis (FHL) with five subtypes (FHL1 to -5), and the related conditions Griscelli Syndrome type 2 (GS2), Hermansky-Pudlak Syndrome type 2 (HPS2) and Chediak-Higashi Syndrome (CHS) (Chediak, 1952; Farquhar and Claireaux, 1952; Griscelli et al., 1978; Hermansky and Pudlak, 1959; Higashi, 1954). Mouse models of these conditions show that upon pathogen challenge, the genetic mutation impairs the secretion of pro-apoptotic factors from CTL (and NK) granules, while the production of cytokines and their release via a different secretory pathway appears to be enhanced (Brisse et al., 2015; de Saint Basile et al., 2015; Jenkins et al., 2015; Reefman et al., 2010). The inability of CTLs and NK cells to clear the infection whilst continuously secreting cytokines promotes the activity of effector immune cells leading to a life-threatening hyper-inflammatory state (haemophagocytic lymphohistiocytosis, HLH) that requires immunosuppressive therapy and ultimately bone-marrow transplantation (Sieni et al., 2014). To date, four FHL-proteins have been identified and a fifth disease-linked genetic locus awaits further investigation (Cote et al., 2009; Feldmann et al., 2003; Ohadi et al., 1999; Stepp et al., 1999; zur Stadt et al., 2009; zur Stadt et al., 2005). The known secretion factors at the CTL immune synapse are the putative vesicle-tether Munc13-4 (FHL3), the soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor

(SNARE) protein Syntaxin 11 (FHL4), the syntaxin-binding protein Munc18-2 (FHL5), the Munc13-4 binding partner Rab27a (GS2), the adaptor protein subunit AP3-beta3A (HPS2) and the lysosomal-trafficking regulator (LYST) protein (CHS) (Barbosa et al., 1996; Barrat et al., 1996; Cote et al., 2009; Dell'Angelica et al., 1999; Feldmann et al., 2003; Fukai et al., 1996; Menasche et al., 2000; Nagle et al., 1996; Perou et al., 1996; zur Stadt et al., 2009; zur Stadt et al., 2005). These proteins are thought to act in successive steps during the maturation, transport and secretion of cytolytic granules (see poster).

HPS2 CTLs that lack AP3 cannot transport their granules to the immune synapse, suggesting that AP3 may coordinate the delivery of a motor protein or a motor-adaptor to the granule membrane (Clark et al., 2003). In GS2, the loss of functional Rab27a means that granules polarise but fail to detach from microtubules and therefore cannot reach the plasma membrane (Haddad et al., 2001; Stinchcombe et al., 2001a). In CTLs, Rab27a functions in granule docking through its interaction with the vesicle tether Munc13-4, whereas in melanoctyes, it has been shown to link to the actin-bound motor MyosinVa to ‘capture’ melanosomes at their target membrane in the cell periphery (Elstak et al., 2011; Hume et al., 2001; Neeft et al., 2005; Shirakawa et al., 2004; Wu et al., 2001).

Munc13-4 associates with the cytolytic granules in attacking CTLs. In Munc13-4-deficient FHL3 CTLs, granules reach the plasma membrane but cannot be ‘primed’ for secretion (Elstak et al., 2011; Feldmann et al., 2003). It has been suggested that in addition to the vesicle tethering interaction with Rab27a, Munc13 proteins also interact with SNARE-complexes, the helical protein bundles that drive membrane fusions, through a MUN-domain (Basu et al., 2005; Guan et al., 2008).

Syntaxin11 and Munc18-2 are binding partners that localise to the plasma membrane of CTL and neutrophils; this strongly suggests that they cooperate to drive the final steps of granule fusion (Brochetta et al., 2008; Cote et al., 2009; Dieckmann et al., 2015; Hackmann et al., 2013; Halimani et al., 2014; Hellewell et al., 2014; zur Stadt et al., 2009). The loss of Syntaxin11 from the plasma membrane of Munc18-2 deficient CTLs supports the notion that Munc18-2 acts as a Syntaxin11 chaperone, similar to Munc18-1 chaperoning Syntaxin1A; however, Munc18-2 was also found to associate with granules in CTLs, mast cells and neutrophils where it may perform a yet unknown function, potentially in association with granule SNAREs (Brochetta et al., 2014; Brochetta et al.,

2008; Dieckmann et al., 2015; Han et al., 2011; Martin-Verdeaux et al., 2003; Rowe et al., 2001).

In CHS, mutation of the LYST protein has been suggested to cause a fission defect which entails the formation of enlarged lysosomes whose excessive size appears to prevent fusion at the immune synapse (Baetz et al., 1995; Durchfort et al., 2012). A recent report showed that overexpression of either Rab27a alone or Rab27a together with Slp3 partially restored granule secretion (measured by surface appearance of the lysosomal membrane protein CD107a), and that co-expression of Rab27a, Munc13-4 and Slp3 rescued the secretion defect of CHS CTLs. This gave rise to the suggestion that LYST might be involved in trafficking of effectors that drive the maturation of perforin-containing vesicles into fully secretion-competent granules (Sepulveda et al., 2015).

Two additional genetic defects have been linked to the immune synapse and can trigger HLH but do not directly affect secretory factors. For instance, FHL2 arises due to loss of the pro-apoptotic factor perforin from the cytolytic granules (Stepp et al., 1999). Interestingly, immune synapses formed by perforin-deficient CTLs and NK cells persist much longer than normal synapses; here the FHL2 CTLs appear to become stuck on the targets they fail to kill (Jenkins et al., 2015). Finally, in X-linked lymphoproliferative disease type 1 (XLP-1), the mutation of the signalling lymphocyte activation molecule (SLAM)-associated protein (SAP) disturbs key intracellular signalling processes that are exerted by SLAM in NK cells and CTLs, thereby resulting in a defective killer response against Epstein-Barr Virus-infected cells (Coffey et al., 1998; Dupre et al., 2005), reviewed by (Tangye, 2014).

Concluding remarks

In this review we have focused on the formation of the CTL immune synapse, which is a highly dynamic process that relies on the close interplay of signalling factors, cytoskeletal elements and membrane fusion machinery to deliver a rapid cytotoxic hit, which allow CTLs to be effective serial killers. There are many more aspects that are being currently explored, including the roles of motor proteins, positive and negative receptor signalling, mechano-sensing and $CD4^+$ cells that acquire cytolytic potential. In addition, the understudied role of the target cell in forming the synapse and the signals that tell the CTL when to depart remain to be uncovered along with a full understanding of how the CTL manages not to kill itself as it releases its deadly cytolytic load.

Summary

This brief review and the poster illustrate the different steps that reveal the specialised mechanisms used to focus secretion at the CTL immune synapse and allow CTL to be such efficient and precise serial killers.

Box 1: TCR signalling in a nutshell

TCR signalling begins with the activating phosphorylation of the kinases Lck and Zeta-chain (TCR)-associated protein of 70kDa (ZAP-70) as Lck associates and phosphorylates the TCR, which promotes the recruitment of ZAP-70 (Chan et al., 1992; Iwashima et al., 1994). In brief, active ZAP-70 phosphorylates tyrosines on linker of activated T cells (LAT) and Src homology 2 domain-containing leukocyte protein of 76kDa (SLP76) to generate the LAT signalosome, a hub for secondary messenger generation (see poster) (Bubeck Wardenburg et al., 1996; Chakraborty and Weiss, 2014; Paz et al., 2001). Associated active phospholipase Cg1 generates two of these messengers, inositol triphosphate (IP3) and diacylglycerol (DAG) (Yablonski et al., 1998). IP3 binds to its ER-associated receptor to induce a global calcium flux, which is crucial for nuclear factor of activated T cells (NFAT) activation, whilst DAG recruits other signaling molecules to the membrane. These include protein kinase C family members (PKCs) that activate integrin activity by phosphorylation of Rap guanine nucleotide exchange factor 2 (RapGEF2), thus activating Rap1; in addition, PKCs control myosin regulatory light chain for efficient MTOC polarisation to the immune synapse (Navarro and Cantrell, 2014; Quann et al., 2011). The signalling of PKCs is thought to be further amplified by their ability to phosphorylate the stabilising loop of protein kinase D2 (PKD2). PKD2 activity enhances transcription of the key cytokines interferon-γ and interleukin-2, as well as promotes Ras activity (Navarro et al., 2014a; Navarro et al., 2014b). Ras is further activated by the action of RAS guanyl nucleotide-releasing proteins (RasGRPs), which themselves are recruited by DAG into close proximity of PKCs, and functions by initiating the mitogen activated protein kinase (MAPK) cascade. This cascade has dramatic effects on CTL metabolism proliferation, transcription, translation and even the microtubule network through the ERK1/2 complex (Navarro and Cantrell, 2014).

References

Azimzadeh, J. and Bornens, M. (2007). Structure and duplication of the centrosome. *J Cell Sci* **120**, 2139-42.

Baetz, K., Isaaz, S. and Griffiths, G. M. (1995). Loss of cytotoxic T lymphocyte function in Chediak-Higashi syndrome arises from a secretory defect that prevents lytic granule exocytosis. *J Immunol* **154**, 6122-31.

Barbosa, M. D., Nguyen, Q. A., Tchernev, V. T., Ashley, J. A., Detter, J. C., Blaydes, S. M., Brandt, S. J., Chotai, D., Hodgman, C., Solari, R. C. et al. (1996). Identification of the homologous beige and Chediak-Higashi syndrome genes. *Nature* **382**, 262-5.

Barrat, F. J., Auloge, L., Pastural, E., Lagelouse, R. D., Vilmer, E., Cant, A. J., Weissenbach, J., Le Paslier, D., Fischer, A. and de Saint Basile, G. (1996). Genetic and physical mapping of the Chediak-Higashi syndrome on chromosome 1q42-43. *Am J Hum Genet* **59**, 625-32.

Basu, J., Shen, N., Dulubova, I., Lu, J., Guan, R., Guryev, O., Grishin, N. V., Rosenmund, C. and Rizo, J. (2005). A minimal domain responsible for Munc13 activity. *Nat Struct Mol Biol* **12**, 1017-8.

Bossi, G. and Griffiths, G. M. (1999). Degranulation plays an essential part in regulating cell surface expression of Fas ligand in T cells and natural killer cells. *Nat Med* **5**, 90-6.

Brisse, E., Wouters, C. H. and Matthys, P. (2015). Hemophagocytic lymphohistiocytosis (HLH): A heterogeneous spectrum of cytokine-driven immune disorders. *Cytokine Growth Factor Rev* **26**, 263-80.

Brochetta, C., Suzuki, R., Vita, F., Soranzo, M. R., Claver, J., Madjene, L. C., Attout, T., Vitte, J., Varin-Blank, N., Zabucchi, G. et al. (2014). Munc18-2 and syntaxin 3 control distinct essential steps in mast cell degranulation. *J Immunol* **192**, 41-51.

Brochetta, C., Vita, F., Tiwari, N., Scandiuzzi, L., Soranzo, M. R., Guerin-Marchand, C., Zabucchi, G. and Blank, U. (2008). Involvement of Munc18 isoforms in the regulation of granule exocytosis in neutrophils. *Biochim Biophys Acta* **1783**, 1781-91.

Bubeck Wardenburg, J., Fu, C., Jackman, J. K., Flotow, H., Wilkinson, S. E., Williams, D. H., Johnson, R., Kong, G., Chan, A. C. and Findell, P. R. (1996). Phosphorylation of SLP-76 by the ZAP-70 protein-tyrosine kinase is required for T-cell receptor function. *J Biol Chem* **271**, 19641-4.

Chakraborty, A. K. and Weiss, A. (2014). Insights into the initiation of TCR signaling. *Nat Immunol* **15**, 798-807.

Chan, A. C., Iwashima, M., Turck, C. W. and Weiss, A. (1992). ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. *Cell* **71**, 649-62.

Chediak, M. M. (1952). [New leukocyte anomaly of constitutional and familial character]. *Rev Hematol* **7**, 362-7.

Clark, R. H., Stinchcombe, J. C., Day, A., Blott, E., Booth, S., Bossi, G., Hamblin, T., Davies, E. G. and Griffiths, G. M. (2003). Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. *Nat Immunol* **4**, 1111-20.

Coffey, A. J., Brooksbank, R. A., Brandau, O., Oohashi, T., Howell, G. R., Bye, J. M., Cahn, A. P., Durham, J., Heath, P., Wray, P. et al. (1998). Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* **20**, 129-35.

Combs, J., Kim, S. J., Tan, S., Ligon, L. A., Holzbaur, E. L., Kuhn, J. and Poenie, M. (2006). Recruitment of dynein to the Jurkat immunological synapse. *Proc Natl Acad Sci U S A* **103**, 14883-8.

Comrie, W. A., Babich, A. and Burkhardt, J. K. (2015a). F-actin flow drives affinity maturation and spatial organization of LFA-1 at the immunological synapse. *J Cell Biol* **208**, 475-91.

Comrie, W. A., Li, S., Boyle, S. and Burkhardt, J. K. (2015b). The dendritic cell cytoskeleton promotes T cell adhesion and activation by constraining ICAM-1 mobility. *J Cell Biol* **208**, 457-73.

Cote, M., Menager, M. M., Burgess, A., Mahlaoui, N., Picard, C., Schaffner, C., Al-Manjomi, F., Al-Harbi, M., Alangari, A., Le Deist, F. et al. (2009). Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest* **119**, 3765-73.

de la Roche, M., Ritter, A. T., Angus, K. L., Dinsmore, C., Earnshaw, C. H., Reiter, J. F. and Griffiths, G. M. (2013). Hedgehog signaling controls T cell killing at the immunological synapse. *Science* **342**, 1247-50.

de Saint Basile, G., Sepulveda, F. E., Maschalidi, S. and Fischer, A. (2015). Cytotoxic granule secretion by lymphocytes and its link to immune homeostasis. *F1000Res* **4**, 930.

Dell'Angelica, E. C., Shotelersuk, V., Aguilar, R. C., Gahl, W. A. and Bonifacino, J. S. (1999). Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol Cell* **3**, 11-21.

Dieckmann, N. M., Hackmann, Y., Arico, M. and Griffiths, G. M. (2015). Munc18-2 is required for Syntaxin 11 Localization on the Plasma Membrane in Cytotoxic T-Lymphocytes. *Traffic* **16**, 1330-41.

Dupre, L., Andolfi, G., Tangye, S. G., Clementi, R., Locatelli, F., Arico, M., Aiuti, A. and Roncarolo, M. G. (2005). SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood* **105**, 4383-9.

Durchfort, N., Verhoef, S., Vaughn, M. B., Shrestha, R., Adam, D., Kaplan, J. and Ward, D. M. (2012). The enlarged lysosomes in beige j cells result from decreased lysosome fission and not increased lysosome fusion. *Traffic* **13**, 108-19.

Elstak, E. D., Neeft, M., Nehme, N. T., Voortman, J., Cheung, M., Goodarzifard, M., Gerritsen, H. C., van Bergen En Henegouwen, P. M., Callebaut, I., de Saint Basile, G. et al. (2011). The munc13-4-rab27 complex is specifically required for tethering secretory lysosomes at the plasma membrane. *Blood* **118**, 1570-8.

Farquhar, J. W. and Claireaux, A. E. (1952). Familial haemophagocytic reticulosis. *Arch Dis Child* **27**, 519-25.

Feldmann, J., Callebaut, I., Raposo, G., Certain, S., Bacq, D., Dumont, C., Lambert, N., Ouachee-Chardin, M., Chedeville, G., Tamary, H. et al. (2003). Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* **115**, 461-73.

Finetti, F., Paccani, S. R., Riparbelli, M. G., Giacomello, E., Perinetti, G., Pazour, G. J., Rosenbaum, J. L. and Baldari, C. T. (2009). Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. *Nat Cell Biol* **11**, 1332-9.

Finetti, F., Patrussi, L., Masi, G., Onnis, A., Galgano, D., Lucherini, O. M., Pazour, G. J. and Baldari, C. T. (2014). Specific recycling receptors are targeted to the immune synapse by the intraflagellar transport system. *J Cell Sci* **127**, 1924-37.

Freeman, S. A., Goyette, J., Furuya, W., Woods, E. C., Bertozzi, C. R., Bergmeier, W., Hinz, B., van der Merwe, P. A., Das, R. and Grinstein, S. (2016). Integrins Form an Expanding Diffusional Barrier that Coordinates Phagocytosis. *Cell* **164**, 128-40.

Fukai, K., Oh, J., Karim, M. A., Moore, K. J., Kandil, H. H., Ito, H., Burger, J. and Spritz, R. A. (1996). Homozygosity mapping of the gene for Chediak-Higashi syndrome to chromosome 1q42-q44 in a segment of conserved synteny that includes the mouse beige locus (bg). *Am J Hum Genet* **59**, 620-4.

Geiger, B., Rosen, D. and Berke, G. (1982). Spatial relationships of microtubule-organizing centers and the contact area of cytotoxic T lymphocytes and target cells. *J Cell Biol* **95**, 137-43.

Goodridge, H. S., Reyes, C. N., Becker, C. A., Katsumoto, T. R., Ma, J., Wolf, A. J., Bose, N., Chan, A. S., Magee, A. S., Danielson, M. E. et al. (2011). Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. *Nature* **472**, 471-5.

Grakoui, A., Bromley, S. K., Sumen, C., Davis, M. M., Shaw, A. S., Allen, P. M. and Dustin, M. L. (1999). The immunological synapse: a molecular machine controlling T cell activation. *Science* **285**, 221-7.

Griscelli, C., Durandy, A., Guy-Grand, D., Daguillard, F., Herzog, C. and Prunieras, M. (1978). A syndrome associating partial albinism and immunodeficiency. *Am J Med* **65**, 691-702.

Guan, R., Dai, H. and Rizo, J. (2008). Binding of the Munc13-1 MUN domain to membrane-anchored SNARE complexes. *Biochemistry* **47**, 1474-81.

Hackmann, Y., Graham, S. C., Ehl, S., Honing, S., Lehmberg, K., Arico, M., Owen, D. J. and Griffiths, G. M. (2013). Syntaxin binding mechanism and disease-causing mutations in Munc18-2. *Proc Natl Acad Sci U S A* **110**, E4482-91.

Haddad, E. K., Wu, X., Hammer, J. A., 3rd and Henkart, P. A. (2001). Defective granule exocytosis in Rab27a-deficient lymphocytes from Ashen mice. *J Cell Biol* **152**, 835-42.

Halimani, M., Pattu, V., Marshall, M. R., Chang, H. F., Matti, U., Jung, M., Becherer, U., Krause, E., Hoth, M., Schwarz, E. C. et al. (2014). Syntaxin11 serves as a t-SNARE for the fusion of lytic granules in human cytotoxic T lymphocytes. *Eur J Immunol* **44**, 573-84.

Han, G. A., Malintan, N. T., Saw, N. M., Li, L., Han, L., Meunier, F. A., Collins, B. M. and Sugita, S. (2011). Munc18-1 domain-1 controls vesicle docking and secretion by interacting with syntaxin-1 and chaperoning it to the plasma membrane. *Mol Biol Cell* **22**, 4134-49.

Hellewell, A. L., Foresti, O., Gover, N., Porter, M. Y. and Hewitt, E. W. (2014). Analysis of familial hemophagocytic lymphohistiocytosis type 4 (FHL-4) mutant proteins reveals that S-acylation is required for the function of syntaxin 11 in natural killer cells. *PLoS One* **9**, e98900.

Hermansky, F. and Pudlak, P. (1959). Albinism associated with hemorrhagic diathesis and unusual pigmented reticular cells in the bone marrow: report of two cases with histochemical studies. *Blood* **14**, 162-9.

Higashi, O. (1954). Congenital gigantism of peroxidase granules; the first case ever reported of qualitative abnormity of peroxidase. *Tohoku J Exp Med* **59**, 315-32.

Hirata, T., Furie, B. C. and Furie, B. (2002). P-, E-, and L-selectin mediate migration of activated CD8+ T lymphocytes into inflamed skin. *J Immunol* **169**, 4307-13.

Hogg, N., Patzak, I. and Willenbrock, F. (2011). The insider's guide to leukocyte integrin signalling and function. *Nat Rev Immunol* **11**, 416-26.

Hume, A. N., Collinson, L. M., Rapak, A., Gomes, A. Q., Hopkins, C. R. and Seabra, M. C. (2001). Rab27a regulates the peripheral distribution of melanosomes in melanocytes. *J Cell Biol* **152**, 795-808.

Iwashima, M., Irving, B. A., van Oers, N. S., Chan, A. C. and Weiss, A. (1994). Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. *Science* **263**, 1136-9.

Jenkins, M. R., Rudd-Schmidt, J. A., Lopez, J. A., Ramsbottom, K. M., Mannering, S. I., Andrews, D. M., Voskoboinik, I. and Trapani, J. A. (2015). Failed CTL/NK cell killing and cytokine hypersecretion are directly linked through prolonged synapse time. *J Exp Med* **212**, 307-17.

Kagi, D., Ledermann, B., Burki, K., Seiler, P., Odermatt, B., Olsen, K. J., Podack, E. R., Zinkernagel, R. M. and Hengartner, H. (1994). Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. *Nature* **369**, 31-7.

Kuhn, J. R. and Poenie, M. (2002). Dynamic polarization of the microtubule cytoskeleton during CTL-mediated killing. *Immunity* **16**, 111-21.

Kupfer, A. and Dennert, G. (1984). Reorientation of the microtubule-organizing center and the Golgi apparatus in cloned cytotoxic lymphocytes triggered by binding to lysable target cells. *J Immunol* **133**, 2762-6.

Kupfer, A., Dennert, G. and Singer, S. J. (1983). Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc Natl Acad Sci U S A* **80**, 7224-8.

Kupfer, A., Dennert, G. and Singer, S. J. (1985). The reorientation of the Golgi apparatus and the microtubule-organizing center in the cytotoxic effector cell is a prerequisite in the lysis of bound target cells. *J Mol Cell Immunol* **2**, 37-49.

Liu, B., Chen, W., Evavold, B. D. and Zhu, C. (2014). Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. *Cell* **157**, 357-68.

Marshall, B. T., Long, M., Piper, J. W., Yago, T., McEver, R. P. and Zhu, C. (2003). Direct observation of catch bonds involving cell-adhesion molecules. *Nature* **423**, 190-3.

Martin-Verdeaux, S., Pombo, I., Iannascoli, B., Roa, M., Varin-Blank, N., Rivera, J. and Blank, U. (2003). Evidence of a role for Munc18-2 and microtubules in mast cell granule exocytosis. *J Cell Sci* **116**, 325-34.

Menasche, G., Pastural, E., Feldmann, J., Certain, S., Ersoy, F., Dupuis, S., Wulffraat, N., Bianchi, D., Fischer, A., Le Deist, F. et al. (2000). Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nat Genet* **25**, 173-6.

Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N. and Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82-6.

Nagle, D. L., Karim, M. A., Woolf, E. A., Holmgren, L., Bork, P., Misumi, D. J., McGrail, S. H., Dussault, B. J., Jr., Perou, C. M., Boissy, R. E. et al. (1996). Identification and mutation analysis of the complete gene for Chediak-Higashi syndrome. *Nat Genet* **14**, 307-11.

Navarro, M. N. and Cantrell, D. A. (2014). Serine-threonine kinases in TCR signaling. *Nat Immunol* **15**, 808-14.

Navarro, M. N., Feijoo-Carnero, C., Arandilla, A. G., Trost, M. and Cantrell, D. A. (2014a). Protein kinase D2 is a digital amplifier of T cell receptor-stimulated diacylglycerol signaling in naive CD8(+) T cells. *Sci Signal* **7**, ra99.

Navarro, M. N., Goebel, J., Hukelmann, J. L. and Cantrell, D. A. (2014b). Quantitative phosphoproteomics of cytotoxic T cells to reveal protein kinase d 2 regulated networks. *Mol Cell Proteomics* **13**, 3544-57.

Neeft, M., Wieffer, M., de Jong, A. S., Negroiu, G., Metz, C. H., van Loon, A., Griffith, J., Krijgsveld, J., Wulffraat, N., Koch, H. et al. (2005). Munc13-4 is an effector of rab27a and controls secretion of lysosomes in hematopoietic cells. *Mol Biol Cell* **16**, 731-41.

Niedergang, F., Di Bartolo, V. and Alcover, A. (2016). Comparative Anatomy of Phagocytic and Immunological Synapses. *Front Immunol* **7**, 18.

Ohadi, M., Lalloz, M. R., Sham, P., Zhao, J., Dearlove, A. M., Shiach, C., Kinsey, S., Rhodes, M. and Layton, D. M. (1999). Localization of a gene for familial hemophagocytic lymphohistiocytosis at chromosome 9q21.3-22 by homozygosity mapping. *Am J Hum Genet* **64**, 165-71.

Paz, P. E., Wang, S., Clarke, H., Lu, X., Stokoe, D. and Abo, A. (2001). Mapping the Zap-70 phosphorylation sites on LAT (linker for activation of T cells) required for recruitment and activation of signalling proteins in T cells. *Biochem J* **356**, 461-71.

Perou, C. M., Moore, K. J., Nagle, D. L., Misumi, D. J., Woolf, E. A., McGrail, S. H., Holmgren, L., Brody, T. H., Dussault, B. J., Jr., Monroe, C. A. et al. (1996). Identification of the murine beige gene by YAC complementation and positional cloning. *Nat Genet* **13**, 303-8.

Potter, T. A., Grebe, K., Freiberg, B. and Kupfer, A. (2001). Formation of supramolecular activation clusters on fresh ex vivo CD8+ T cells after engagement of the T cell antigen receptor and CD8 by antigen-presenting cells. *Proc Natl Acad Sci U S A* **98**, 12624-9.

Prosser, S. L. and Morrison, C. G. (2015). Centrin2 regulates CP110 removal in primary cilium formation. *J Cell Biol* **208**, 693-701.

Purbhoo, M. A. (2013). The function of sub-synaptic vesicles during T-cell activation. *Immunol Rev* **251**, 36-48.

Quann, E. J., Liu, X., Altan-Bonnet, G. and Huse, M. (2011). A cascade of protein kinase C isozymes promotes cytoskeletal polarization in T cells. *Nat Immunol* **12**, 647-54.

Reefman, E., Kay, J. G., Wood, S. M., Offenhauser, C., Brown, D. L., Roy, S., Stanley, A. C., Low, P. C., Manderson, A. P. and Stow, J. L. (2010). Cytokine secretion is distinct from secretion of cytotoxic granules in NK cells. *J Immunol* **184**, 4852-62.

Ritter, A. T., Asano, Y., Stinchcombe, J. C., Dieckmann, N. M., Chen, B. C., Gawden-Bone, C., van Engelenburg, S., Legant, W., Gao, L., Davidson, M. W. et al. (2015). Actin depletion initiates events leading to granule secretion at the immunological synapse. *Immunity* **42**, 864-76.

Rowe, J., Calegari, F., Taverna, E., Longhi, R. and Rosa, P. (2001). Syntaxin 1A is delivered to the apical and basolateral domains of epithelial cells: the role of munc-18 proteins. *J Cell Sci* **114**, 3323-32.

Sepulveda, F. E., Burgess, A., Heiligenstein, X., Goudin, N., Menager, M. M., Romao, M., Cote, M., Mahlaoui, N., Fischer, A., Raposo, G. et al. (2015). LYST controls the biogenesis of the endosomal compartment required for secretory lysosome function. *Traffic* **16**, 191-203.

Shirakawa, R., Higashi, T., Tabuchi, A., Yoshioka, A., Nishioka, H., Fukuda, M., Kita, T. and Horiuchi, H. (2004). Munc13-4 is a GTP-Rab27-binding protein regulating dense core granule secretion in platelets. *J Biol Chem* **279**, 10730-7.

Sieni, E., Cetica, V., Hackmann, Y., Coniglio, M. L., Da Ros, M., Ciambotti, B., Pende, D., Griffiths, G. and Arico, M. (2014). Familial hemophagocytic lymphohistiocytosis: when rare diseases shed light on immune system functioning. *Front Immunol* **5**, 167.

Soares, H., Lasserre, R. and Alcover, A. (2013). Orchestrating cytoskeleton and intracellular vesicle traffic to build functional immunological synapses. *Immunol Rev* **256**, 118-32.

Stepp, S. E., Dufourcq-Lagelouse, R., Le Deist, F., Bhawan, S., Certain, S., Mathew, P. A., Henter, J. I., Bennett, M., Fischer, A., de Saint Basile, G. et al. (1999). Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* **286**, 1957-9.

Stinchcombe, J. C., Barral, D. C., Mules, E. H., Booth, S., Hume, A. N., Machesky, L. M., Seabra, M. C. and Griffiths, G. M. (2001a). Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J Cell Biol* **152**, 825-34.

Stinchcombe, J. C., Bossi, G., Booth, S. and Griffiths, G. M. (2001b). The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* **15**, 751-61.

Stinchcombe, J. C., Majorovits, E., Bossi, G., Fuller, S. and Griffiths, G. M. (2006). Centrosome polarization delivers secretory granules to the immunological synapse. *Nature* **443**, 462-5.

Stinchcombe, J. C., Randzavola, L. O., Angus, K. L., Mantell, J. M., Verkade, P. and Griffiths, G. M. (2015). Mother Centriole Distal Appendages Mediate Centrosome Docking at the Immunological Synapse and Reveal Mechanistic Parallels with Ciliogenesis. *Curr Biol* **25**, 3239-44.

Tangye, S. G. (2014). XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol* **34**, 772-9.

Tanos, B. E., Yang, H. J., Soni, R., Wang, W. J., Macaluso, F. P., Asara, J. M. and Tsou, M. F. (2013). Centriole distal appendages promote membrane docking, leading to cilia initiation. *Genes Dev* **27**, 163-8.

Tschopp, J. and Nabholz, M. (1990). Perforin-mediated target cell lysis by cytolytic T lymphocytes. *Annu Rev Immunol* **8**, 279-302.

Ueda, H., Morphew, M. K., McIntosh, J. R. and Davis, M. M. (2011). CD4+ T-cell synapses involve multiple distinct stages. *Proc Natl Acad Sci U S A* **108**, 17099-104.

Vivar, O. I., Masi, G., Carpier, J. M., Magalhaes, J. G., Galgano, D., Pazour, G. J., Amigorena, S., Hivroz, C. and Baldari, C. T. (2016). IFT20 controls LAT recruitment to the immune synapse and T-cell activation in vivo. *Proc Natl Acad Sci U S A* **113**, 386-91.

Wheatley, D. N. (1995). Primary cilia in normal and pathological tissues. *Pathobiology* **63**, 222-38.

Wu, X., Rao, K., Bowers, M. B., Copeland, N. G., Jenkins, N. A. and Hammer, J. A., 3rd. (2001). Rab27a enables myosin Va-dependent melanosome capture by recruiting the myosin to the organelle. *J Cell Sci* **114**, 1091-100.

Yablonski, D., Kuhne, M. R., Kadlecek, T. and Weiss, A. (1998). Uncoupling of nonreceptor tyrosine kinases from PLC-gamma1 in an SLP-76-deficient T cell. *Science* **281**, 413-6.

Yi, J., Wu, X., Chung, A. H., Chen, J. K., Kapoor, T. M. and Hammer, J. A. (2013). Centrosome repositioning in T cells is biphasic and driven by microtubule end-on capture-shrinkage. *J Cell Biol* **202**, 779-92.

zur Stadt, U., Rohr, J., Seifert, W., Koch, F., Grieve, S., Pagel, J., Strauss, J., Kasper, B., Nurnberg, G., Becker, C. et al. (2009). Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. *Am J Hum Genet* **85**, 482-92.

zur Stadt, U., Schmidt, S., Kasper, B., Beutel, K., Diler, A. S., Henter, J. I., Kabisch, H., Schneppenheim, R., Nurnberg, P., Janka, G. et al. (2005). Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet* **14**, 827-34.