F 3 Biophysics of Killing

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Contents

Lecture Notes of the 49^{th} IFF Spring School "Physics of Life" (Forschungszentrum Jülich, 2018).

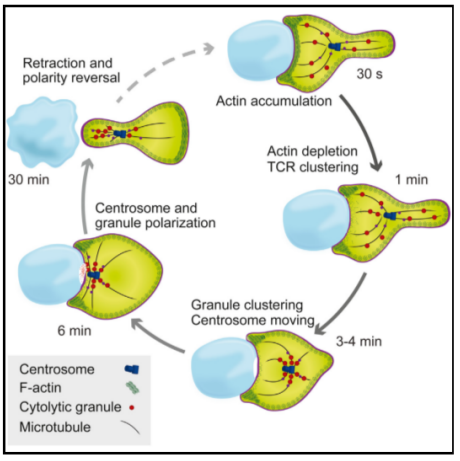
1 Introduction

Immunology is a huge field that covers the study of the immune systems in all organisms [1]. Every one of us has an immune system and it saves our live by fighting hostile intruders, pathogenes, causing infections in our body. Cells of the innate immune system respond recognize and respond to pathogens in a generic way and provide an immediate defense against infection but do not provide long-lasting immunity to the host. The adaptive immune system can recognize novel pathogens (say viruses) which have never before infested our body and develop strategies for their destruction. Moreover it can develop memories of earlier infections and can thus rapidly recognize and fight pathogens intruding our body for the second time.
We will not endeavor to give an overview over the wide area of immunology in a single lecture, we refer to "Janeway's Immunobiology" [1] or the corresponding chapters of Alberts [2]. Instead we will focus here on the crucial aspect in an immune response: the actual elimination or killing of pathogen-infected or tumorigenic cells. The main cytotoxic killer cells of the human body to achieve this are the natural killer (NK) cells from the innate immune system and cytotoxic T-lymphocytes (T cells) from the adaptive immune system. Common to both is that they form a tight contact, the immunological synapse (IS), with targets and release their lytic granules containing perforin/granzyme and cytokine containing vesicles via exocytosis - reminiscent of synaptic contacts and neurotransmitter release from one neuron to another. Although T cells can also kill via non-directional release of cytotoxic material the "kill-shot" via IS formation is most frequent and effective.

Successful killing involves the following stages:

- activation of the T cell (not necessary for NK cells)
- target search in an inhomogeneous environment
- recognition of the target upon contact
- polarization and establisment of the IS
- delivery of the cytotoxic material

The sketch on the right (from [17]b) illustrates the events from target recognition to killing, in particular the establishment of an immunological synapse (IS).



In each stage physical processes can be identified that we aim to elucidate in this lecture. In the following we treat these stages in separate sections, each providing a brief overview over its biology and biophysics.

2 Friend or foe: how to recognize an unknown enemy

A central problem to be solved by the immune system is to discriminate between the organism's own healthy cells and tissue – and so to avoid harming its own organism by "friendly fire" – and virus-infected, tumor or intruders. For completeness we give a brief and simplified summary of the fascinating story of friend or foe discrimination in the immune system – an introduction with a physics perspective is [3], the full details of the current biomolecular understanding can be found in [1]. A key player on the target side is the major histocompatibility complex (MHC), which is a set of cell surface proteins, which is essential for the adaptive immune system to

recognize foreign molecules, which in turn determines histocompatibility. Natural killer (NK) cells as part of the innate immune system recognize the absence of MHC complex on the surface of a cell, for example bacteria, which they will then eliminate. T cells on the other hand check the MHC complex for signs of foreign cell material as follows:
The MHC complex binds to antigens (here peptides, i.e. amino acid chains) derived from the cell owning the MHC and display them on the cell surface for recognition by the appropriate T cells. Each MHC molecule on the cell surface displays a molecular fraction of one of the proteins continually synthesized and degraded within the cell, called an epitope. In its entirety the MHC population on the cell surface indicates the balance of proteins within the cell. Virus-infected or tumor cells display antigens that do not belong to the epitope repertoire of the healthy organism and can thus be recognized by T cells expressing the corresponding T cell receptor (TCR) able to bind to this antigene. The population of naive T cell express a broad palette of antigen specific T cell receptors which can recognize a substantial number of distinct antigene-MHC-complexes. Due to this diversity only a few from the $\mathcal{O}(10^{12})$ T cells of the human body are expected to bind to a particular non-self antigen - estimates are that about one T cell in 10^5-10^6 T cells is specific for a given antigen [4].
The immune repertoire, i.e. the diversity of immune cell receptors that exist in T cells (note that each T cell expresses only a single receptor kind), is generated during the formation of the T cell in the thymus: it is the outcome of a remarkable process in which germline DNA is edited to produce a repertoire of T cells with varied antigen receptor genes [1]. The process is called VDJ recombination because the germline contains multiple versions of so-called V- (for ”variable”), D- (for ”diversity”), and J- (for ”joining”) genes, particular instances of which are quasi-randomly selected, stochastically edited, and joined together to produce a new surface receptor gene each time a new immune system cell is generated. Following that, the repertoire is edited in the thymus. Cells whose protein would cause an immune reaction with the body, are removed. Cells which actually detect an invading organism, become more numerous. And cells with new types, may be added. The statistical distribution of these biochemical events (and the resulting receptor coding sequences) has recently be inferred from the large T cell sequence repertoires that are becoming available via high-throughput sequencing technology [5] providing insight into the molecular mechanisms involved [6].

3 Activation and formation of the immunological synapse

In contrast to NK cells a naive population of T-lymphocytes needs to be stimulated by specific antigen presenting cells (APCs), such as dendritic cells (DC) or macrophages. DCs posses different mechanisms to internalize extracellular material (phagocytosis, makropinocytosis, virus infection, transfer from other DCs) hence they can present antigens from practically all pathogens. When they do this, for instance in the micro-environment of an inflammatory site, they wander into the lymphatics. In the lymph nodes they present their surface to naive T cells and act as so-called antigen presenting cells (APCs). Once a naive T cell with the appropriate T cell receptor (TCR) encounters a DC with the corresponding MHC-antigene complex a tight contact between DC and T cell, a immunological synapse (IS), is established. At the IS signaling events take place (also involving calcium as a second messanger as discussed in section 6 below) that will activate the T cell (see [7] for a review), which means that it will proliferate and search for target cells presenting the antigen that fits its TCR.
The specific binding of the MHC-antigen complex to the TCR leads to the recruiting of several

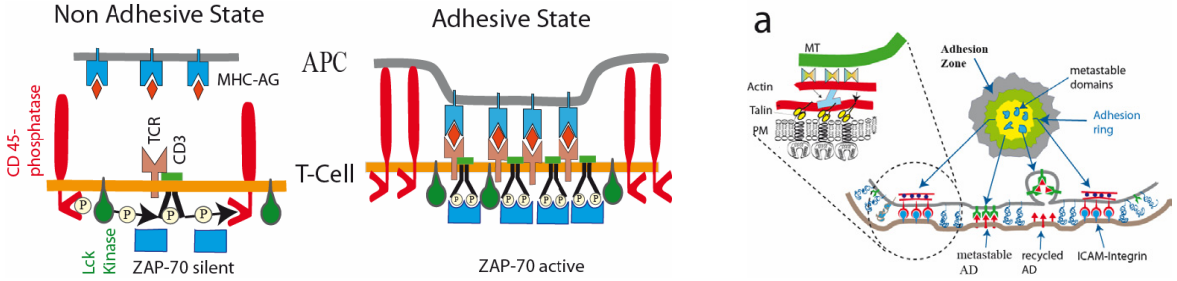


Fig. 1: Left: *Model of activation of T cells by micro-clusters formed during the initial phase of T cellAPC encounters (i.e. before the formation of SMAC). The 70kDa kinase ZAP-70 is activated by binding to the phosphorylated tyrosine groups of the CD3 ζ-chains.* **Center**: *abolishment of ZAP-70 activation by CD45-mediated continuous dephosphorylation of CD3. Right side: formation of active immune synapse (IS) by lateral segregation of bound TCRMHCpeptide pairs, resulting in the expulsion of the inhibitor CD45.* **Right:** *Model of the formation of the peripheral adhesion zone (such as p-SMAC) serving in the long-time stabilization of cellcell contacts enclosing a central zone (such as a c-SMAC) in which signalling micro-domains of tight adhesion formed by MHCAG binding (left side) could coexist with areas where mature TCRCD3 complexes are taken up by endocytosis (right side). The inset on the left shows the coupling of the MT plus end to the actin cortex by MTactin binding proteins. From [8].*

other membrane-bound helper molecules (such as CD4 or CD8, where CD stands for ”cluster of differentiation”). This results in the generation of micro-domains of tight adhesion that are composed of a mixture of the receptor-ligand pairs (formed between MHC-antigen and the TCR) and the helper molecules (see [8] for more details). The adhesion of the T cell on the APC leads to the assembly of proteins involved in T cell activation into micro-domains (also called adhesion domains) from which proteins counteracting the activation (such as CD45) are expelled by steric forces, see Fig. 1. This activation of the T cell by numerous adhesion domains formed within the contact zone is reminiscent of the activation of nerve or muscle cells by synapses contacting the target cell, which motivated their name immunological synapses.
In a second phase of the T cell - APC contact establishment, long-lived global reaction spaces (called supramolecular activation complexes (SMAC)) form by talin-mediated binding of the T cell integrin (LFA-1) to the counter-receptor ICAM-1, resulting in the formation of ring-like tight adhesion zones (peripheral SMAC), c.f. Fig. 1 right). The adhesion domains move to the center of the intercellular adhesion zone forming the central SMAC, which serve in the recycling of the adhesion domain by down-regulation of the signaling by internalization (endocytosis) of the TCR-CD3 complexes. In [8] a model is proposed for the formation of p- and c-SMAC and the Mexican hat-like adhesion caps formed at the IS. Similar global reaction platforms are formed by killer cells to destruct target cells (see section 5 and 6 below), which implies that in this way closed reaction spaces for the localized secretion of lytic proteins, such as perforin, are formed, avoiding the loss of toxic material into the surrounding extracellular space.

4 Target search and environmental influence

During an immune response a T cell has to encounter its cognate antigen many times in different contexts and tissues which necessitates their high motility. The movement of T cells in the lymph nodes has been well studied with two photon microscopy, which showed that

they indeed migrate very fast with average speeds of 10 $\mu m/min$ and peak speeds of up to 25 $\mu m/min$ [9]. They are highly versatile migrators that can effectively navigate almost any tissue of the body. As other leukocytes, T cells migrate using an amoeboid mode of locomotion in three-dimensional in vivo environments. Characteristic for this T cell migration mode is a rounded but polarized morphology with frequent and rapid extensions of pseudopodia at the leading edge. Those are driven by the polymerization of filamentous actin (F-actin) that extends the plasma membrane in the direction of migration. The lagging tail contains stable F-actin networks rich in myosin motors and undergoes little overall deformation. In order to adapt to the varying tissue architecture and molecular composition of their surrounding T cells can rapidly alternate between adhesion-dependent and adhesion-independent motility. During adhesion-driven migration the cell is anchored to the cell to the substrate by integrin-mediated attachments in the extended pseudopodia and the subsequent forward displacement of the cell is driven by the retrograde flow of treadmilling actin filaments coupled to the extracellular matrix (for the underlying biochemical and mechanical processes see chapter C6 on the active cytoskeleton). The contractile lagging tail is only loosely adherent and actomyosin-mediated forces help detach mature adhesion sites and propel the cell body forward. In non-adhesive environments, leading edge pseudopodia are wedged into confined spaces in the extracellular matrix, which, coupled with actomyosin contraction of the rear, allows for a concerted translocation of the cell.

The principal purpose of T cell motility is to search for cognate antigen on APCs and for target cells. The process of search is a fundamental requirement in almost any biological system in which many agents (that is, cells and organisms) reside within an ecosystem much larger than their perceptual capabilities [10]. As with many biological movements, T cell motility was initially characterized as resembling a random walk or diffusion [11]. Two different types of random migration have been used to model T cell motility: diffusive (Brownian-type) random walks [11] and superdiffusive (Lévy-type) random walks [12]. When pauses drive the overall movement, subdiffusive patterns may also be observed. In addition, under some circumstances and for short times, T cells can undergo fully ballistic migration (that is, in a nearly straight line). This range of T cell motility is generated by a combination of cell-intrinsic locomotion events (for example, those controlled by rates of actin polymerization and location of cortex contraction), physical guidance cues from the microenvironment (for example, cues provided by collagen fibres and the orientation of stromal cells) and chemical information provided by the microenvironment (for example, chemokines, antigen dose and through co-stimulatory molecules) [10].

In a pathological scenario, not all cells in a given patrolling area are necessarily target cells. For example, NK cells encounter stromal cells, infiltrated immune cells, as well as malignant cells with expression of MHC class I molecules. These bystander cells pose a challenge to NK cells to efficiently identify their targets in a complex microenvironment. Whether and how the presence of bystander cells can affect the efficiency for NK cells to find and kill their targets has been investigated in [13]. There it has been shown that the presence of non-target bystander cells unexpectedly enhance the killing efficiency as well as NK cell migration. In Fig. 2a-c the analysis of a simple reaction-diffusion model for target search in an obstacle park shows that obstacles generally decrease the search efficiency. Thus the observation of an increase in search efficiency in the presence of bystander cells implies that they cannot be just obstacles but must somehow accelerate the search of the NK cells. In Fig. 2d a corresponding simplified reaction diffusion model is sketched in which bystanders are surrounded by an circular area in which the diffusion constant of the searchers is increased. 2e-g shows the analysis of this model and

supports quantitativley the prediction that the assumed local acceleration of the NKs close to bystander indeed leads to a search efficiency increase.

When the NK migration speed was experimentally measured and a comparison between speeds close to and far away from bystanders were made it was observed that NK motility close bystanders was significantly increased. It turned out that bystander cells increase the H_2O_2 concentration in the surrounding environment, which is known to have an accelerating effect on the migration of killer cells [13]. Thus bystander cells could be used to manipulate the microenvironment of killer cells searching for targets increasing their search efficiency.

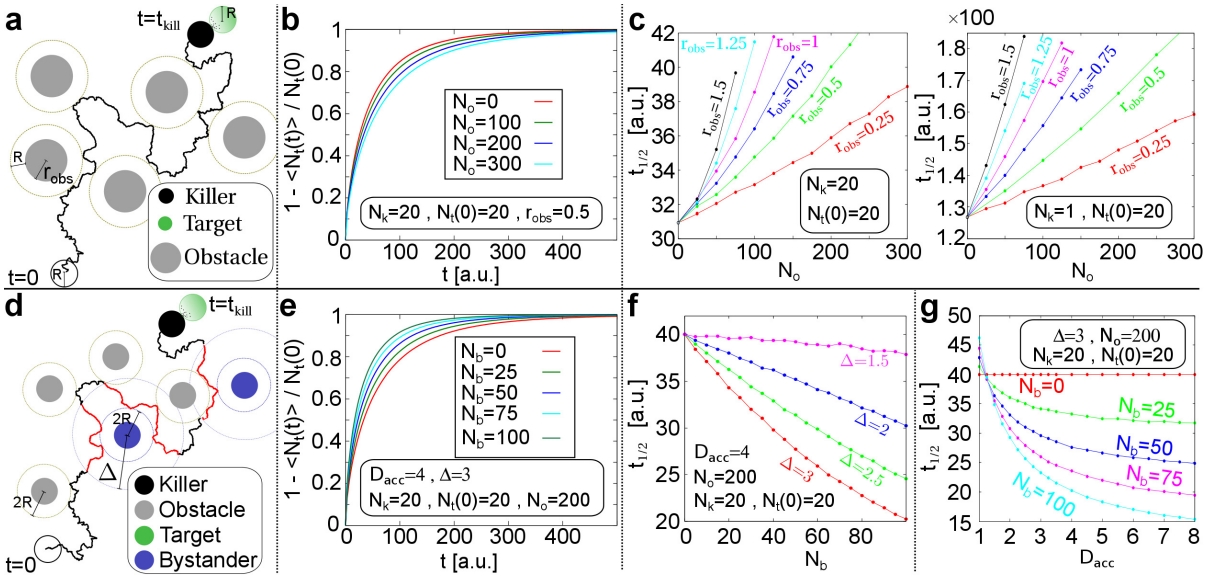


Fig. 2: **(a)** *Sketch of the mathematical model of a Brownian (diffusive) disk-like particle performing a random search for disk-like targets among disk-like obstacles: the black disk represents one randomly moving NK cell, the black wiggly lines its diffusive motion with diffusion constant D, grey disks the immobile obstacles of radius robs, and green disks immobile targets of radius R. Targets that are hit, here at time* $t_{\rm kill}$*, are removed upon contact with the NK cells.* **(b)** *The ratio of killed targets is depicted as a function of time for* $N_k = 20$ *killers,* $N_t(0) = 20$ *targets, different numbers of obstacles* N_o*, and* $r_{\rm obs} = 0.5$*.* **(c)** *The average half time* $t_{1/2}$ *is given as a function of the number of obstacles* N_o *for different values of robs for the same number of killers and targets (left) and for the case of only one killer (right).* **(d)** *Same as (a) but now with bystanders (blue). Diffusion of the* N_K *cells is accelerated by an increased diffusion constant,* $D_{\rm acc} > D$*, within the circles with radius* Δ *around bystanders, as indicated by red portions of the wiggly lines representing the random killer motion. Otherwise the symbols are similar to (a).* **(e)** *The ratio of killed targets for the model shown in (d) as a function of time for* $N_k = 20$ *killers,* $N_t(0) = 20$ *targets,* $N_o = 200$ *obstacles,* $D_{\rm acc} = 4$ *and* $\Delta = 3$ *and different numbers of obstacles.* **(f)** *Average half time* $t_{1/2}$ *as a function of* N_b *for* $N_k = 20$ *killers,* $N_t(0) = 20$ *targets,* $N_o = 200$ *obstacles and* $D_{\rm acc} = 4$ *and different values of* Δ*.* **(g)** *Average half time* $t_{1/2}$ *as a function of* $D_{\rm acc}$ *for* $N_k = 20$ *killers,* $N_t(0) = 20$ *targets,* $N_o = 200$ *obstacles and* $\Delta = 3$ *and different numbers of obstacles.From [13]*

5 MTOC relocation during T cell polarization

Once a NK or T cell has identified a target cell (see section 2) and established a contact zone (see section 3) one observes a re-polarization of the cell involving the rotation of the microtubule (MT) spindle and a movement of the centrosome or microtubule organizing center (MTOC), the organelle in which the MT fibers of the cytoskeleton are anchored, to a position that is just underneath the plasma membrane at the center of the IS [14, 15, 19] (c.f. Fig. 3). Concomitantly a massive relocation of organelles attached to MTs is observed, including the Golgi apparatus [16] lytic granules (the vesicles containing the cytotoxic material) [17] and mitochondria [18]. As a consequence granules come closer to the point where their cytotoxic content can be release towards the target (see section 7) and mitochondria modulate the redistribution of calcium in the cell leading to increased cytosolic calcium concentrations necessary for signaling during activation and killing (see section 6).

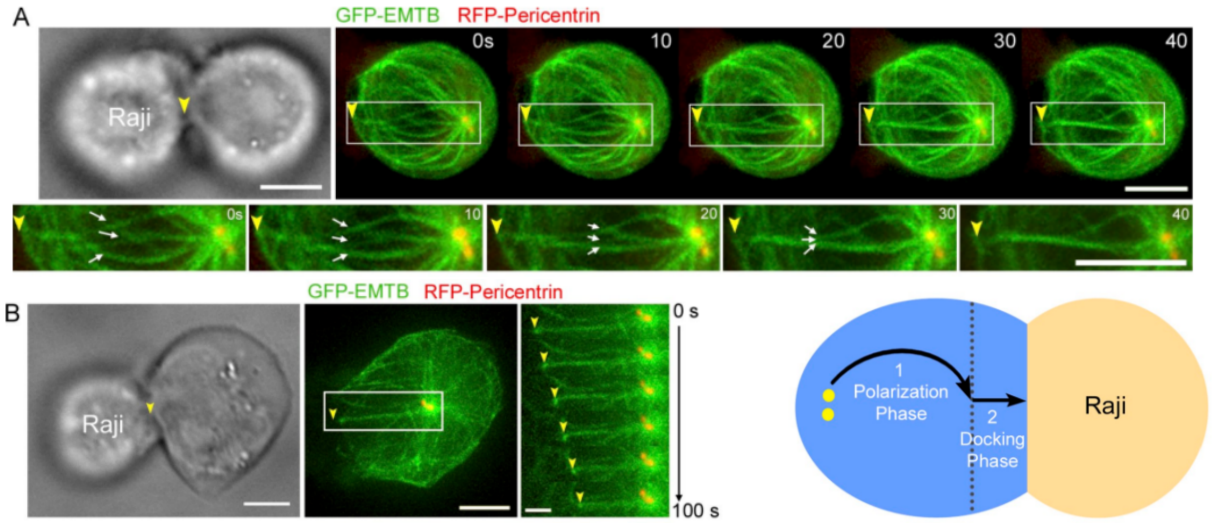


Fig. 3: **(A)** *Time-lapse images of a JurkatRaji conjugate (transmitted image) in which the Jurkat has been labeled with 3GFP-EMTB to mark MTs and RFP-Pericentrin to mark the MTOC (which appears yellow) (fluorescent images). Zoomed images of the boxed regions in the fluorescent images are shown underneath. The white arrows point to the straightening MTs, and the yellow arrowheads point to the center of the IS where these MTs abut.* **(B)** *As in A, but including a kymograph demonstrating the shortening of the straight MT stalk.* **(C)** *Cartoon depicting the two apparent kinetic phases.From [19]*

Experiments suggest the massive relocation of the MTOC is driven by dynein, the molecular motors that move along MTs towards the minus end (towards the MTOC). According to this hypothesis, repositioning is driven by a cortical sliding mechanism involving dynein motors anchored at the IS that reel in the centrosome by pulling on MTs that pass over the interface [15]. A theoretical model analyzing the cortical sliding mechanism has been developed and analyzed in [20], see Fig. 4. The cell outline consists of an unattached round part and of a flat part which is attached to the target cell (called synapse, or synaptic plane). The large nucleus is coupled to the aster of MTs converging near its surface, and the mobility of both is constrained by the cell outline. MTs slide along the cell outline in the areas of contact with the targets. This active sliding drives all movements that are observed. The movements are opposed by MT bending elasticity and by viscous drag in the cytoplasm [20]. The model reproduces

qualitatively the motion of the MT-network together with large fluctuations of the centrosome next to the cell-cell interface and fluctuations between interfaces with simultaneously engaged targets as observed experimentally in [15].
The experiments of [19], displayed in Fig. 3 examined in closer detail the dynamic organization of the MT cytoskeleton during MTOC re-positioning. Immediately after conjugation of the T cell with the APC one observes several MTs that project from the centrosome toward the APC (Fig. 5A, inset, white arrows, 0s frame) and that terminate in end-on fashion at the approximate center of the IS (Fig. 5A, compare yellow arrowheads in the phase and fluorescent images). Over the next 30s, these MTs straighten and come together to form an apparent bundle of MTs (referred to as the MT "stalk") that terminates in end-on fashion near the center of the IS (follow the white arrows in the insets). Most importantly, MT stalks were then observed to undergo shortening (Fig. 5B; follow the yellow arrowhead marking the point of contact of the stalk with the IS relative to the position of the yellow centrosome), causing the IS and centrosome to approach each other (in this case the IS interface came toward the centrosome). It could be shown that shortening MT stalks pulled the centrosome all the way to the IS and that such perpendicular, centrally anchored, shortening MT stalks coincident with MTOC repositioning [19]. These results are consistent with MTOC repositioning being driven by a capture-shrinkage mechanism focused at the center of the IS and not with the cortical sliding mechanism operating at the IS periphery, c.f. Fig. 4.

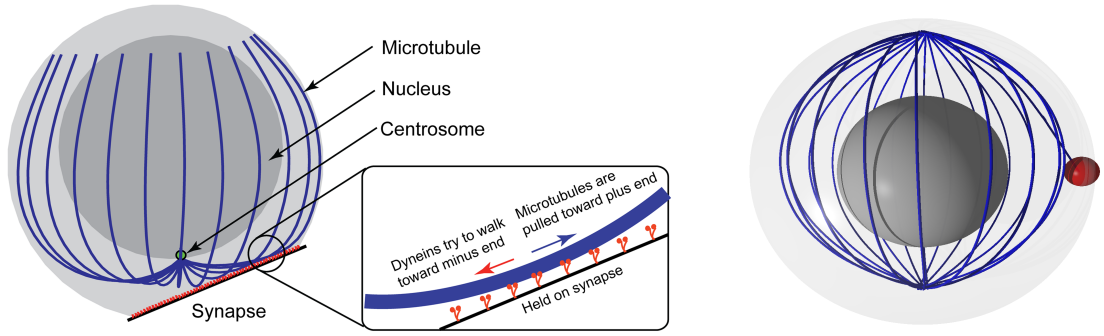


Fig. 4: Left: *Sketch of the cortical sliding model. MTs (modelled as semi-flexible filaments, are blue, dynein motors anchored at the IS are red. From [20].* **Right:** *Corresponding sketch of the capture end-on shrinkage mechanism. Red indicates the point in the IS where the end of a MT bundle is captured and pulled by dynein.*

It is a force dependent depolymerization rate of MTs that realizes the chemo-mechanical shrinkage of the MTs when their plus end is pulled against the cell membrane by dynein, as has been demonstrated recently in vitro [21]. It was also observed that MTOC repositioning involves two distinct kinetic phases comprising a fast (3.3μm/min) polarization phase and a slower (0.9μm/min) docking phase, with a transition between them occurring at 2.2μm from the IS. The biomechanical origin of the observed biphasic repositioning is still elusive.

6 Calcium dynamics during T cell polarization

Calcium (Ca^{2+}) is one of the most important intracellular and intercellular messengers. It transmits signals that arrive at the cell surface to intracellular targets via the transient increase of the intracellular concentration. Information is often transmitted as a Ca^{2+}wave travelling through

the cytoplasm of a cell or a group of cells. $Ca^{2+}$is a simple ion which cannot transmit information by its binding specificity or simply by its presence. Consequently, the signal is encoded in temporal and spatial patterns very similar to the use of electric voltage or current signals in information technology [22]. A comprehensive review on the physics of Ca^{2+}signaling can be found in [22].
Several of the signaling steps governing target killing through T cell and/or NK cells are Ca^{2+}dependent. 1) MTOC relocalization to the IS [19]; 2) mitochondria relocalization to the IS [18]; 3) secretion of LG at the IS [23]; 4) perforin-dependent lysis of target cells [24]. Ca^{2+}signaling in NK and T cells is initiated by binding of the TCR to to its cognate antigen (see section 2 and 3). The following signaling cascade leading to Ca^{2+}release from the endoplasmatic reticulum (ER) is quite common in eukaryotic cells (see [22]): The TCR stimulates PLCγ1 activity that generates IP_3, which diffuses to the ER where it binds to Ca^{2+}permeable ion channel receptors (IP_3R) on the ER membrane leading to the release of ER Ca^{2+}into the cytoplasm (see Fig. 5A for a sketch). What comes next is characteristic for NK and T cell Ca^{2+}dynamics: Depletion of ER Ca^{2+}triggers a sustained influx of extracellular Ca^{2+}through the activation of plasma membrane Ca^{2+}release-activated Ca^{2+}(CRAC) channels in a process known as store-operated Ca^{2+}entry (SOCE). This SOCE has to be sustained during T cell activation.

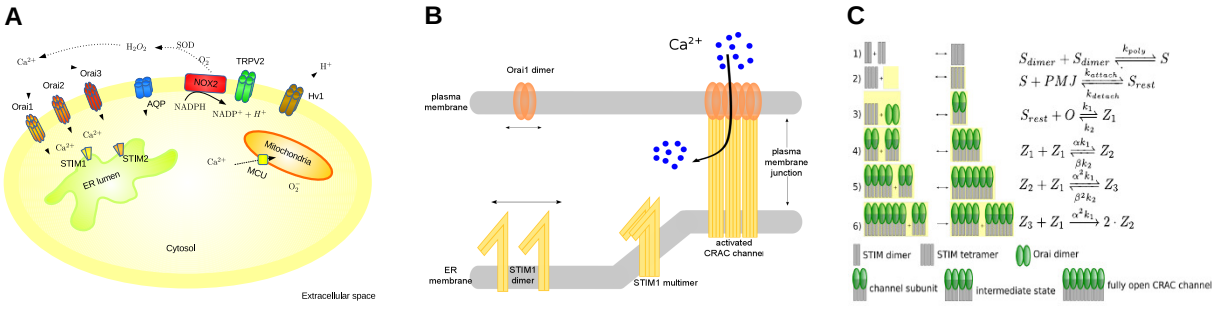


Fig. 5: **(A)** *Schematic representation of ion channels, the ER with the STIM proteins and the mitochondria.* **(B)** *Schematic representation of CRAC / Orai channel formation, After ER store depletion STIM1 proteins multimerize and move and attach to the PM junction where they trap Orai1 dimers to form CRAC channels and to mediate the Ca^{2+}influx.* **(C)** *Schematic representation of the reaction scheme underlying CRAC / Orai channel formation . Reaction 1 describes the STIM multimerization, reaction 2 the attachment of these multimers to the PM. Reactions 3 to 5 represent the stepwise CRAC channel formation. Reaction 6 is a additional reaction between CRAC channel subunits and fully open CRAC channels.*

For decades the CRAC channels had only been identified by their biophysical properties, and it has just been in the past few years that the pore-forming subunit of the channels was identified as the four-transmembrane domaincontaining molecule Orai1. Additionally, studies in the past few years have revealed the sensor for depleted ER Ca^{2+}stores and the activator of CRAC channels as stromal interaction molecule (STIM). STIM is an ER-resident transmembrane protein which has a Ca^{2+}binding site within the ER lumen, serving as Ca^{2+}sensor, and a domain that can bind to Orai when the Ca^{2+}binding site in the ER lumen is empty. Thus the drop in ER luminal $Ca^{2+}$is sensed by STIM, which undergo a conformational change, multimerize and move to regions near the plasma membrane, so called plasma membrane junctions (PMJ). Here STIM1 proteins trap Orai1 ion channel proteins diffusing within the plasma membrane and depending on the STIM1-Orai1 stochiometry different Orai1 conductance states are reached open

and selectively conduct Ca^{2+}ions into the cell (see Fig. 5B). A quantitative analysis of these membrane bound events leading to SOCE has been perfromed in [27] based on a a reaction-diffusion model with reaction schemes as indicated in Fig. 5C.

Ca^{2+}dynamics during activation of T cells is particularly interesting, because it involves a sustained increase of intracellular Ca^{2+}levels that results in the activation of $Ca^{2+}$and calmodulin-dependent transcription factors hat in turn activate a variety of transcription programs. Activated calcineurin dephosphorylates members of the nuclear factor of activated T cells (NFAT) family, leading to their translocation to the nucleus resulting in differential gene expression patterns, which eventually transform the naive T cell into a killer. The duration of the Ca^{2+}increase necessary for a succesfull completion of these steps (up to 1 hour) is remarkable, because Ca^{2+}pumps and Ca^{2+}buffers in living cells keep the cytosolic Ca^{2+}level very low (ca. 80 nanomolar, as compared to, for instance, Ca^{2+}concentration in mineral water which is a few millimolar, i.e. 10^6 times higher). The interesting question is, how T cells succeed to outmaneuver its Ca^{2+}removing machinery to sustain 10 fold increased Ca^{2+}concentrations for up to 1 hour.

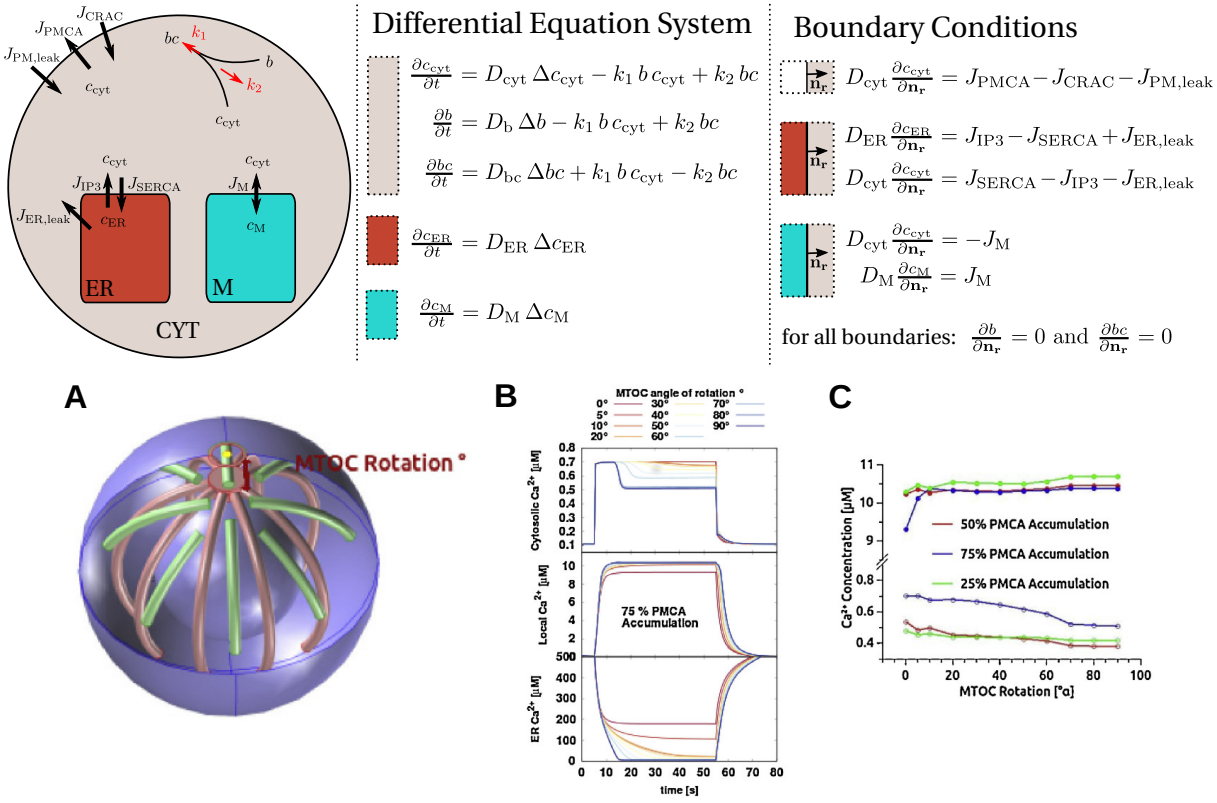


Fig. 6: Top: *Sketch of the reaction-diffusion model for the intracellular Ca^{2+}dynamics in T cells represented by the Ca^{2+}fluxes between the different compartments: cytosol, mitochondria, ER, and extracellular space. Bottom: Ca^{2+}concentrations depend on mitochondria ratation angle - model prediction with working SERCAs and spindle ER geometry.* **Bottom: (A)** *Sketch of the mitochondria (green) and a spindle ER geometry (red).* **(B)** *Time course of Ca^{2+}concentrations in the cytosol (top), at the IS (middle), and in the ER (bottom) for the geometry in A. CRAC channels are activated at t = 10s and deactivated at t = 60s.* **(C)** *Plateau value (at t = 40s) of cytosol (global) and IS (local) Ca^{2+}concentrations as a function of the rotation angle of the mitochondrial network for different ratio of PMCA accumulation at the IS. From [28].*

It turned out that a key factor for the sustained increased cytosolic Ca^{2+}concentration is the relocation of mitochondria towards the IS [26], which are powerful Ca^{2+}buffers and can distribute Ca^{2+}within the cytsol via a filamentous network. The reason for the mitochondria relocation was identified to be the cytoskeleton rotation discussed in the previous section 5 [28]: mitochondria attached to MTs (via molecular motors, by which they can also move along MTs) are simply dragged with the moving cytoskeleton. Activated Orai channels also accumulate at the IS such that it appears plausible that mitochondira modulate the strength of the Ca^{2+}influx. However, since activated Orai channels are directly linked (via STIM) to the ER, it is questionable that mitochondria can come closer than 100-200 nanometer to the Orai channel, which impedes a direct modulation of channel inhibition [26]. Since plasma membrane calcium ATPase (PMCA) pumps (which expel $Ca^{2+}$from the cytosol against a concentration gradient into the extracellular space) also accumulate at the IS [26] an alternative explanation has been proposed in [27, 28]: the mitochondria absorb the Ca^{2+}entering the IS microdomain through Orai/CRAC channels before it can be extruded again by the PMCA clusters in the IS. With the help of a mathematical model for the intracellular Ca^{2+}dynamics it was indeed shown in [28] that the repositioning of mitochondria alone can modulate the global cytosolic Ca^{2+}concentration, independent of any influence of mitochondrial position on CRAC/Orai channel activity.
A sketch of the model is shown in Fig. 6: it consists of a reaction-diffusion model for the intracellular Ca^{2+}dynamics summarized in the top panel of Fig. 6 and a specific geomtry for various cell compartmens (cell body, mitochondria, ER) and location of the CRAC channels shown in Fig. 6A. The assumed geometry of mitochondria is inspired by the fact that they form filaments along MTs, which themselves are arranged in a spindle-like geometry (c.f. 3). Results for the local (at the IS) and global (cytosolic) Ca^{2+}concentration are shown in Fig. 6B and C and demonstrate that global Ca^{2+}concentration increases with decreasing rotation angle of the cytoskeleton network, i.e. with decreasing distance of mitochondria from the IS.

7 Vesicle delivery and intracellular search strategies

In order to kill the target cell by NKs and T cells the lytic granules have to be transported to the IS where they can dock and release their cytotoxic content via exocytosis. The cytoskeleton of living cells is a self-organizing filamentous network that shapes the mechanical and rheological characteristics of the cell and coordinates cargo transport between different cellular regions (see also chapters C3, C4, C5 and C6). Intracellular transport of particles equipped with one or several motors [30] switches between two modes of motion: free diffusion within the cytosol and ballistic motion during intermittent bindings of the motor(s) to a cytoskeleton filament. Typically cargo, like proteins, vesicles, and other organelles, is produced or emerges in one region of the cell and is needed in some other region or has to fuse with a reaction partner being produced somewhere else. In the absence of a direct connection between origin and destination the transport is a stochastic process [31] with random alternations between ballistic and diffusive motion, which is denoted as intermittent search [32]. A particular set of parameters defining the stochastic process, like the switching rate between ballistic and diffusive transport, represents an intermittent search strategy – and it has been shown that an optimal choice of this parameter enhances the search efficiency in a homogeneous and isotropic environment, i.e. under the assumption of a constant density of filaments with no preferred direction [33].
Real cell cytoskeletons display a complex spatial organization, which is neither homogeneous nor isotropic. For instance in cells with a centrosome, like NK and T cells, the microtubules

emanate radially from the microtubule organizing center (MTOC) and actin filaments form a thin cortex underneath the plasma membrane with broad distribution of directions again centered around the radial direction (c.f. Fig. 7a). Since the MTOC is frequently located close to the nucleus, transport of cargo between the plasma membrane and the nucleus, as for instance necessary for the establishment of synaptic junctions, appears to be facilitated by this cytoskeleton organization. In essence the specific spatial organization of the cytoskeleton filaments represents, in connection with intracellular transport, an intermittent search strategy, probably highly efficient for specific frequently occurring tasks, but less well suited for others. In [34, 35] the efficiency of spatially inhomogeneous intermittent search strategy for particular task has been analyzed and compared with homogeneous search strategies with the help of a model for intermittent dynamics in spatially inhomogeneous environments. Three basic search tasks frequently encountered in intracellular transport have been investigated in [34, 35]:
1) Transport of cargo from an arbitrary position within the interior of the cell, typically from a location close to the nucleus, to a specific area on the cell boundary, the plasma membrane. The transport of lytic granules to the IS involves the cytoskeleton [17] and represents such a task. The stochastic search for a specific small area on the boundary of a search domain is reminiscent of the so-called narrow escape problem [36].
2) The enhancement of the reaction kinetics between two reaction partners by motor assisted ballistic transport. It has already been demonstrated that spatially homogeneous and isotropic intermittent search strategies can be efficient, but can only realized in those parts of a biological cell, where cytoskeleton filaments are homogeneously and isotropically distributed, which is certainly not true for the whole cell body.
3) Finally the combination of the reaction and escape problem, where cargo has first to bind to a reaction partner before it can be delivered or dock at a specific area in the cell boundary as, for instance, a synapse. A prominent realization is the docking of lytic granules at the IS that requires the pairing with CD3 endosome beforehand [29]. Single lytic granules have a low docking probability at the IS, whereas endosomes containing CD3 molecules have a high docking probability. Apparently it represents an advantageous strategy to guarantee the delivery of cytotoxic cargo exclusively to the IS via binding to these endosomes in advance.
A search strategy that idealizes the cytoskeleton structure in a spherical cell of radius R consists of microtubule filaments emanating radially from the MTOC in the cell center and randomly oriented actin filament in a cortex of width Δ underneath the plasma membrane, as sketched in Fig. 7. Mathematically such a filament distribution is defined by the probability density $\rho(\Omega, r)$ to choose direction Ω conditional on the switch from the diffusive to a ballistic mode at position r and can, for simplicity, be parameterized by a width $\Delta \in [0, R]$ and a probability $p \in [0, 1]$: In the central region, $0 < |r| < R - \Delta$, $\rho(\Omega, r) = p$ for radial outward transport, i.e. Ω is the direction of the position vector, and $\rho(\Omega, r) = 1 - p$ for radial inward transport; and in the periphery, $R - \Delta < |r| < R$, $\rho(\Omega, r) = 1/4\pi$ uniform for all Ω. The intracellular motion of cargo is modeled by an intermittent search process [32], in which a particle per-forms random motion in two alternating modes: Brownian motion with a diffusion coefficient D, and ballistic motion with velocity v. Transitions between the two modes occur stochastically with rates k and k', respectively, see Fig. 7 for a sketch in case of the narrow escape problem, and deterministically from ballistic to diffusive at $|r| = 0$, $R - \Delta$, and R.
The efficiency of a search strategy, or a specific directional distribution, $\rho(\Omega, r)$, is measured in terms of the time that a searcher needs on average until it hits the target the first time, the so-called mean first passage time (MFPT). A major progress in the analytical determination of the MFPT in 2d and 3d bounded domains was made in [37] with a computational method that is

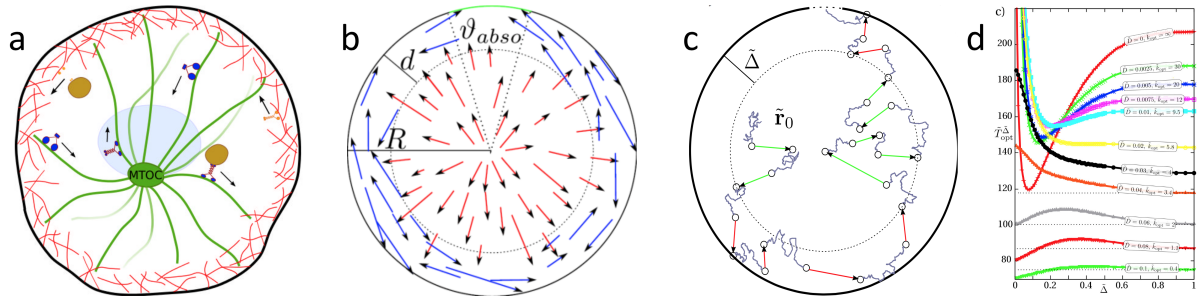


Fig. 7: **(a)** *Sketch of the cellular cytoskeleton with microtubules in green and actin filaments in red.* **(b)** *Sketch of the idealized direction distribution, red only radial transport, blue transport in all directions, green the narrow escape region.* **(c)** *Sketch of the random intermittent search process with the idealized direction distribution, Δ=thickness of the idealized actin cortex. Green radial ballistic transport, grey wiggly lines: diffusive motion, red ballistic transport in arbitrary directions, =thickness of the idealized actin cortex.* **(d)** *Mean first passage time (MFPT) for the narrow escape problem as a function of the actin cortex thickness in the random intermittent search process sketched left. For small diffusion constants the MFPT has a pronounced minimum between $\Delta/R = 0$ and 0.2 corresponding to a thin actin cortex. From [34].*

based on an expression for the MFPT between two nodes of a general graph developed in [38]. Still a spatially inhomogeneous problem like the one defined by above appears analytically intractable, although some regions in the parameter space may be approximately treatable analytically by combining the known results for homogeneous searches. Numerically it was found in [34, 35] that the confinement of randomly oriented cytoskeleton filaments to a thin actin cortex is not a handicap for the cell, but can actually, in conjunction with radial transport along microtubules, substantially increase the efficiency of transport tasks. For the narrow escape problem, which is exemplarily shown in Fig. 7D, it turns out to be a superior strategy to allow only radial outward ballistic transport from the center towards a thin sheet of thickness Δ underneath the boundary, where ballistic transport in all directions is possible. This thin boundary layer allows an accelerated random motion along the boundary to find the escape region, somewhat reminiscent of purely diffusive search with an accelerated surface mediated diffusion [39]. A similar result holds for the reaction kinetics problem, in which the target is not located on the boundary: here again optimal strategies with small thickness Δ exist, in particular better than the homogeneous strategy [36], but the optimal probability for forward/backward radial transport is now around p = 1/2. This result is reminiscent of an acceleration of purely diffusive search kinetics by following boundaries with an increased diffusion constant [40]. The reaction-escape problem combines both scenarios and the optimal forward/backward radial transport probability depends on the size ratio of target and escape region.

8 Summary

We have discussed various biophysical processes involved in the successful killing of pathogen-infected or tumorigenic cells by NK or T cells: the self-organized arrangement of membrane proteins into characteristic patterns at the IS, the mechanical relocation of the MT cytoskeleton towards the IS by molecular motors, the modulation of the cytosolic calcium concentration by

mechanically moving compartments, and the delivery of lytic granules to the IS. Important open questions and issues of ongoing research concern for instance the role of actin in particular and the forces in general in the formation of the IS and the bio-mechanics of the different migration modes of NK and T cells during their search for targets in different environments. Of particular interest here is the question how the cell processes signals from the environment and transforms them into mechano-chemical events connected with the cell cytoskeleton that lead to directional changes during migration and shape and polarization changes upon contact with a target cells. It would be highly desirable to understand the ways in which environmental factor influence the efficiency NK and T cells in searching and killing target cells since it bears the potential to improve the immune response of an organism in fighting a disease or cancer.

Acknowledgements: I thank my collaborators Markus Hoth, Bin Qu and Barbara Niemeyer from the Biophysics Department of the Medical School of the Saarland University as well as my students Anne Hafner, Barbara Schmidt, Karsten Schwarz, Ivan Hornak and Thierry Fredrich for their valuable inputs to this lecture and to my understanding of the biophysics of killing.

Abbreviations:

APC	antigen presenting cell
CRAC	calcium release activated channel
CTL	cytotoxic T lymphocyte
DC	dendritic cell
ER	endoplasmatic reticulum
MFPT	mean first passage time
IS	immunological synapse
LG	lytic granule
MHC	major histocompatibility complex
MT	microtubule
MTOC	microtubule organizing center
NFAT	nuclear factor of activated T cells
NK	natural killer (cell)
PM	plasma membrane
PMJ	plasma membrane junction
PMCA	plasma membrane calcium ATPase
SMAC	supramolecular activation complex
SOCE	store operated calcium entry
STIM	stromal interaction molecule
TCR	T cell receptor

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