**This is a postprint of an article published in
Reichardt, P., Gunzer, M.
The biophysics of T lymphocyte activation in vitro and in vivo
(2006) Results and Problems in Cell Differentiation, 43, pp. 199-218**

"Cell Communication in Nervous and Immune System"

Chapter: "The biophysics of T lymphocyte activation in vitro and in vivo"

Running title: Biophysics of T cell activation

Peter Reichardt; Matthias Gunzer

Junior Research Group of Immunodynamics, German Research Centre for Biotechnology (GBF), Mascheroder Weg 1, D-38124 Braunschweig,

Address correspondence to Dr. Matthias Gunzer, Immunodynamics, German Research Centre for Biotechnology (GBF), Mascheroder Weg 1, D-38124 Braunschweig, Germany

Office: 49 (0)531-6181-977

Fax : 49 (0)531-6181-312

Email: mgunzer@gbf.de

Keywords: T cell activation, DC, cell-cell interaction, Biophysics

Document Word Count: 5.164

Document Character Count (with spaces): 31.716

N° of References: 85

Abstract

T cell activation is crucial for the development of specific immune reactions. It requires the physical contact between T cells and antigen-presenting cells (APC). Since these cells are initially located at distinct positions in the body, they have to migrate and find each other within secondary lymphoid organs. After encountering each other both cells have to maintain a close membrane contact sufficiently long to ensure successful signaling. Thus, there is the necessity to temporarily synchronize the motile behavior of these cells. Initially, it had been proposed, that during antigen recognition T cells receive a stop signal and maintain a stable contact with APCs for several hours when an appropriate APC has been encountered. However, direct cell observation via time-lapse microscopy in vitro and in vivo has revealed a different picture. While long contacts can be observed, many interactions appear to be very short and sequential despite efficient signaling. Thus, two concepts addressing the biophysics of T cell activation have emerged. The single encounter model proposes that after a period of dynamic searching a T cell stops to interact with one appropriately presenting APC until signaling is completed. The serial encounter model suggests that T cells are able to collect a series of short signals by different APC until a critical activation threshold is achieved. Future research needs to clarify the relative importance of short and dynamic versus long-lived T-APC encounters for the outcome of T cell activation. Furthermore, a thorough understanding

of the molecular events underlying the observed complex motility patterns will make these phenomena amenable for intervention, which might result in the identification of new types of immune modulating drugs.

<271 words>

1. Introduction

T lymphocytes are positioned at the crossroads of the human immune system. They are central for efficiently mounting an adaptive immune response. To fulfill their tasks, T cells undergo a complex maturation process and display a characteristic migratory pattern where they physically and functionally interact with many cell types of the body. Thus, for proper functioning of the immune system, the ability to migrate in an autonomous fashion is an indispensable prerequisite [1]. In case of the *de novo* induction of a specific immune response, two diverse cell types, a T cell and an antigen-presenting cell (APC: mainly dendritic cells (DC), B cells or macrophages (M ϕ)) have to physically engage and need to exchange specific signals. T cells recognize a complex of a peptide antigen in combination with an MHC molecule on the surface of APC via their antigen specific T cell receptor (TCR). During thymic development, the clonotypic TCRs are generated by a chance recombination of a huge number of genetic building blocks, also including mechanisms to add variety. This is done by each developing T cell individually, until a functioning TCR is obtained. Thus, although each T cell expresses only one type of TCR, the diversity of the TCRs in the whole T cell compartment is immense [2,3]. However, although there are millions of different TCR, each one is able to recognize a spectrum of peptide-MHC complexes with different affinity, thus, each antigen will be recognized by a number of individual T cells, albeit with distinct efficiency. This system may help finding a balance between the ability to recognize a broad set of antigens and to mount a specific immune response in a short enough time [2].

Only contacts with peptide-MHC complexes of sufficiently high TCR affinity will ultimately lead to an activation associated change in one or both partners, e.g. blast formation and proliferation in T and B cells or antibody production and antibody class switch in B cells, only. However, all cell types, that need to interact initially reside in very diverse areas of the

body. Moreover, only very few of a body's antigen-presenting cells will carry the specific antigen looked for by a specific T cell. In contrast to hormonal signaling, which allows communication between far apart cells by means of small, transportable molecules, from all we know, it is not possible to communicate between an APC and a T cell, which antigen has entered the body, other than by direct physical cell-cell contact. We will see later how, despite these obstacles, the body ensures efficient T cell activation leading to a successful immune response by creating unique immunological "marketplaces" of intense cell communication and cell-cell contact, the lymph nodes. To better understand the functional importance of the migration and cell-cell interactions of T lymphocytes, we will first follow a developing T cell along its way from the bone marrow to the thymus, where precursor T cells get prepared for their central task, the encounter of a foreign antigen and the appropriate response to it. The activation of T cells by antigen occurs in the lymph nodes, the anatomy of which we will therefore briefly discuss. Mapping the T cell's workplace will then open the ground for having a closer look at experimental data obtained from direct observations of cell-cell interactions and at the mechanistic models generated from these data trying to explain how cellular contacts will enable a T cell to get activated in vivo.

2. The role of T cells within the immune system - a T cell biography

2.1 T cell Selection in the Thymus

Like all hematopoietic cells T lymphocytes originate in the bone marrow. As T cell precursors, they leave the bone marrow via the bloodstream to begin a rigorous two-step test for functionality and self-destructive potential in the thymus termed positive and negative selection. Details of the thymic selection processes have been described in a number of excellent reviews [3-5]. Very briefly, thymocytes must try to establish contact via their then only half-completed TCR with MHC molecules on specialized cortical thymic epithelial cells and DCs, respectively, which both present a large variety of self peptides. During these interactions the T cell precursors are sorted by the strength of signals resulting from binding of their TCR to the peptide-MHC complexes presented in the thymus [6, 7]. T cells are positively selected as a result of a weak TCR signal, whereas no signal results in death by neglect and a too strong signal results in negative selection [8]. For effective interactions with stromal cells thymocytes decrease their motility, and establish sustained calcium oscillations that may enhance the expression of genes enabling positive selection [9]. The signaling strength mediated by the TCR is believed to be the key to both positive and negative selection. Thus, T cells are selected for survival, if the signals received from antigen-presenting DCs residing in the medulla of the thymus do not exceed a certain threshold. If

they do, suspicion is raised that this high affinity towards the selecting peptide might pose a risk for this T cell to later develop into an auto-reactive T-cell thereby priming the organism for the development of auto-immune disease. Consequently, the potentially dangerous T cell is driven into apoptosis. The duration and strength of signal also participates in the decision to which of the two main T cell lineages a particular T cell will belong to in the future: to the CD8⁺ (killer) T cells bearing direct cytotoxic potential or to the CD4⁺ (helper) T cells providing support to CD8 cells for killing, to B cells for producing antibodies and to macrophages/monocytes to kill intracellular pathogens such as listeria [10]. While there is evidence that positive and negative selection occur spatially distinct from each other and consecutively in time [6, 11], both processes are aimed at achieving a single goal, namely to provide the highest possible number of T cell specificities available for fighting invaders while minimizing the risk of attacking self tissue. This process is rigorous, more than 90 percent of all T lymphocytes entering the thymus fall prey to it and die without ever leaving the thymus [12].

2.2 T cell Circulation and Homeostasis

At the end of the thymic selection process, the now mature naïve (i.e. antigen-inexperienced) T cells are released into the blood and circulate through the periphery [13]. This process comprises the migration of T cells through secondary lymphoid organs and peripheral nonlymphoid tissues. The persistent recirculation is fundamental to the maintenance of immune surveillance. A variety of proteins on the T cell surface facilitates their interaction with resident endothelial, epithelial, stromal and immune cells and with matrix components of secondary lymphatic organs such as spleen, lymph nodes, and the lymphoid tissue of the respiratory tract and intestine [13-15]. Initially, naïve T cells will randomly enter secondary lymphoid organs (e.g. lymph nodes) [16, 17]. Upon scanning the environment for a cognate antigen, T cells will predominantly encounter self peptides with which they undergo serial antigen-unspecific, short-lived contacts providing sub-threshold survival signals and being implicated in T cell homeostasis [17a]. It could be shown that recirculating naïve T cells constitutively contact high numbers of DC as they migrate through the perifollicular regions of the lymph node [49]. The lymph node is also the place where the most important cell-cell interactions occur with respect to pro-inflammatory T cell activation towards antigens of invading organisms. Here, antigen drained from the site of infection/inflammation arrives within afferent lymphatic vessels, in soluble form or bound to antigen presenting cells, mostly DC [18]. The close proximity in the lymph node of foreign antigens, APC and T cells is essential for the transformation of a resting naïve T cell into an activated, armed effector T lymphocyte.

2.3 T cell activation in the lymph node – a marketplace analogy

In order to get activated T cells need to find APC presenting the correct peptide-MHC complexes on their surface. However, there exists an intrinsic information problem. Neither does a T cell have any knowledge, whether the antigen for which its TCR is specific has been taken up somewhere in the body. Nor does a DC know, which T cell is specific for the pathogen that it just had phagocytosed and whose processed peptide antigens are now

presented on its MHC molecules. Likewise, a B cell cannot tell, which T cell can provide help for the antigen, that fits into its B cell receptor (BCR), a membrane bound version of the antibody, that is being produced by this particular B cell.

The way nature has solved this problem is probably best explained by a town's marketplace analogy. A marketplace is a defined region where all town's inhabitants go to check, what is being offered by the merchants. Merchants go to this place and set up their booths because they know, that they will have a very high chance to get in contact with the town's inhabitants. Inhabitants entering have a preference for a specific good, but they don't know who is selling this particular product. Therefore, they have to migrate over the entire market place and interact with many merchants to check the goods. As a result, two individuals, who did not know of each other's existence before and were initially far apart meet and interact fruitfully.

Transferred to the immune system, the marketplace is a lymph node, the merchants would be APC and the customers would be T cells. The "goods" that are being presented are self and foreign peptide antigens, which are loaded onto MHC molecules of the APC. Thereby powerful APCs such as DCs, which carry $1-8 \cdot 10^6$ MHC complexes on their surface [19], most likely present several hundred if not thousands of different peptides and thus can serve more than one distinct T cell clone (Fig. 1, [19, 20]).

However, it is not only the T cell that moves towards the lymph node, DCs may travel long ways, too. DC usually reside in peripheral tissues of the body and form a tight network of gatekeepers for invading foreign antigen (Fig. 2, [21]).

Fig. 1: The lymph node as busy marketplace for T cell activation and B cell help.

Fig. 2: Langerhans cells, the dendritic cells of the skin, form a tight meshwork of cells in the epidermis of a mouse ear.

They leave their position once they have taken up a sample of this antigen by phagocytosis and start a migration process, that ends when the DC reaches the lymph node draining the area of infection, usually 1-2 days later [18]. This process has been particularly well documented for Langerhans cells (LC), the DC of the epidermis [22]. Activating tissue resident LC, e.g. by painting contact sensitizers on the skin or simply by explanting skin and putting it in culture, induces the rapid emigration of skin resident LC into the lymph vessels of the dermis [23] and from there into draining lymph nodes [24].

The lymph node architecture comprises an inner medullary area rich in plasma cells and macrophages and an outer cortical region. The node is surrounded by a sinus and then a capsule. Afferent lymphatics drain into this sinus, thereby bringing new antigens to the node for presentation to T-cells [18, 25-27]. An unstimulated node's cortex contains primary follicles which are mainly composed of B cells and some DC surrounded by a DC rich T cell zone. Lymphocytes entering the lymph node from the blood stream exit the blood vessels via crossing the walls of specialized post-capillary or high endothelial venules (HEV), around which DCs may line up in strategic positions to immediately interact with newly arriving T cells [28, 29]. In fact, DCs will be the major antigen presenters as they are also the most effective professional antigen-presenting cells (APC) [30]. However, B cells may also actively participate in antigen presentation, if they were able to internalize soluble antigen via their B cell receptor, process the antigen and load the generated peptides onto MHC molecules and then transport these MHC/peptide complexes to the cell surface [31]. B cells will then migrate with directional preference toward the B-zone-T-zone boundary by upregulating the chemokine receptor CCR7, which enables the B cells to sense a CCR7-ligand gradient, which extends from the T zone into the border of the B cell follicle [32, 33]. Immigrant T cells will start scanning the surrounding APCs for a peptide/MHC complex capable of binding with high affinity to their TCR. By doing this, they display an interesting and diverse spectrum of biophysical interactions with each of these types of APC [28, 34], which will be looked at in detail below. If the search is finally successful, the T cell will respond to the new situation with proliferation and differentiation into a primed effector cell. For CD4⁺ T cells, this would be a T helper cell whose main function is to support B cells, whose presented peptides are recognized, to produce antibodies corresponding to the protein, from which these peptides were derived. Another important function of helper cells is to support the generation of cytotoxic effector cells. Consequently, a part of the pre-activated CD4⁺ T cell will march towards the lymph node's B cell zone [32], while others stay in the T cell zone or leave the lymph node to function in the periphery [38]. A CD8⁺ cell, in contrast, would turn into a cytotoxic T cell (CTL) possessing various direct and indirect possibilities of killing infected cells. In the much more frequent case that a T cell will fail to meet an appropriate antigenic partner, the T cell will leave the lymph node and re-start circulating the body [13].

2.4 Homing of activated T cells

Following a successful activation, T cells differentiate into effector cells with a certain tissue-specific phenotype guiding their homing preferences [13, 35-37]. Key factors that collectively determine the homing of leukocytes are interactions between integrins or selectins with their tissue adhesion molecules as well as interactions between chemokine receptors and their specific ligands, the chemokines [13, 35-37]. In addition, the presence of inflammatory stimuli modulates the homing behavior of immune cells [38]. Activated T cells start homing to peripheral tissues where they exert pro-inflammatory regulatory effector functions or recirculate as memory cells [39]. At the target location, T cells leave the vessels and enter the local tissue ready to exert their effector function [13, 13a]. Alternatively, antigen primed T cells might become memory cells, an option which may occur early during T cell development as well as after differentiation into an effector cell [40]. Memory cells either reside centrally in lymphoid tissues or in the periphery with a very high likelihood to expand rapidly upon re-encounter of the stimulating antigen [41].

2.5 T cell Regulation

A special case emerges when the T-cell/APC contact does not generate a full T-cell activation status aimed at performing the optimal immune response, but rather results in T cells suppressing the immune response. It is currently a matter of debate, whether a regulatory cell is a differentiation status of any given T cell or rather a specific type of T cell, that is preferentially expanded in situations where suboptimal activation is prevailing. Evidence for naturally occurring regulatory cells has been obtained [42]. But antigen presentation by immature and semimature DC *in vivo* might lead to incomplete T cell activation and de novo induction of regulation [42, 43]. Regulatory T cells have attracted great interest in recent years and certainly play a very important role in maintaining an efficient and safe immune system [44, 45]. The role of cell-cell interactions during this process has not yet been studied in detail [46].

3. The biophysics of T cell activation

The direct microscopic observation of T cells revealed that upon migration T cells (which appear round in liquid cell culture) show a very dynamic cell shape with clear polarity: a tail is formed by a motile uropod, as well as a head consisting of the cytoskeletal leading edge by which cell-cell contacts are initiated (Fig. 3, [28, 47-50]).

Fig. 3: A naïve T cell (green) contacting an antigen specific naïve B cell (orange). Note the intense cell-cell contact as well as the membrane protrusions of the T cell engaging the B cell. The fibrous structures are protein strands consisting of collagen type 1, which was used to simulate a 3-D environment. A dynamic aspect of such cell pairs can be obtained from the supplemental material of [34] on the webpage of the BLOOD journal.

When a firm contact is established, the T cell can give up its migratory morphology and round up again or remain stationary [51, 52]. However, it may also maintain a dynamic state of motility and crawl along the APC surface with substantial velocities of up to 10 μm per minute [28, 34, 49, 53]. How long will such individual contacts persist *in vivo* and where are they formed during an immune response? In the following we will now have a closer look at the available experimental data and on the models of T cell activation derived from these experiments.

3.1 Classical in vitro studies of T-APC interaction

Initially, due to the absence of better technology, researchers were only able to take a static picture of the T-APC interaction. Classical experiments from Kupfer and colleagues showed, that antigen-specific T cells physically interacted with B-cells presenting the relevant peptide antigen. Thereby the microtubule organizing center (MTOC) of both cells became reoriented towards each other [54]. It was the same group that 9 years later showed that upon interaction the two cells formed a highly organized multi-molecular assembly of proteins at the interaction plane that is now being referred to as the immunological synapse (IS) [55]. At the same time it was demonstrated in liquid culture, that naïve T cells need a certain period of time in contact with APC to reach their full effector potential. This was $\sim 6\text{h}$ for the best possible APC, but could be as long as 30 h for weak APC. Thereby, powerful APC. Like DC were able to induce T cell proliferation within a few hours of contact, even at low doses of antigen, while weak APC such as B cells needed long contact times, even at high levels of pMHC occupancy [56]. In addition, the first approaches using live cell imaging of T cells migrating on lipid bilayers found that in the absence of recognizable peptide-MHC complexes T cells were highly mobile and migrated on ICAM-1 layers [57]. However, when in addition to ICAM-1, a specific peptide-MHC complex was available within the lipid bilayers, the T cells stopped moving and remained at the MHC-peptide containing spot [57].

3.2 The single encounter model of T cell activation

The above data were used to establish a model of T cell activation in lymph nodes stating that naïve T cells are actively motile in lymphoid tissues thereby scanning the surface of all

available APC for specific peptide-MHC complexes. However, as soon as a high affinity (cognate) MHC-peptide complex is discovered, T cells stop and remain at this location, forming a stable cell contact with the APC for several hours until full activation is reached. During that time, an immunological synapse is formed which is essential for signaling and which is possibly also involved in stopping T-cell motility [58, 59]. The concept of a cognate cell pair stopping and thus stabilizing its contact was appealing as it provided a solution to the apparent problem that *in vivo* a rather low number of motile peptide-loaded APC had to activate an also very limited number of motile T cells [2] specific for an invading pathogen. It appeared logical that in the rare event of a specific APC hitting a specific T cell this interaction had to be stabilized in order to maximize the chance of a successful T cell activation. Thus, the complete stopping of the two cellular partners was considered a general rule for all situations of specific T cell activation.

3.3 Challenges introduced by live cell imaging experiments

This concept, however, was challenged by the first observations employing live cell imaging of antigen presentation in liquid cultures with T cell blasts and with macrophages serving as APC. Here it was found that T cells migrate over APC bodies stopping only for a limited amount of time on one APC but then resume motility and wander on to the next APC. From these observations it was concluded that a T cell sequentially could gain signals from more than one APC [60]. However, one point of critique regarding these experiments was that the particular behavior of the T-cells used in this study could result from their pre-activated state and thus might not reflect the behavior of a naïve, non-activated T-cell. Therefore, in a new approach, naïve T cell receptor (TCR) transgenic T cells plus peptide-loaded highly activated DC were used as antigen-specific activation pair and the two types of cells were embedded in a 3D collagen matrix as approximation of the 3D environment present within lymphatic tissue. Using this experimental system, it was shown that it was a general feature of naïve T

cells to form contacts with specific antigen-loaded DC only transiently, for a few minutes rather than hours (Fig. 4, [53]).

Fig. 4: T cells (green) interacting with an antigen specific DC (orange) within 3-D collagen. Not the huge size of the DC relative to the T cells as well as the ability of the DC to “serve” three T cells at the same time. In vivo, DC can contact up to 200 cells at any given time point [49].

In addition, T cells were observed to engage multiple DC in a serial fashion. It was assumed that although each of these contacts was able to induce signals within the T cell, none of these short-lived contacts was sufficient to activate the T cell but rather a sequence of several contacts was needed [53]. It could later be shown that T cells may indeed gradually increase the level of signaling intermediates [61] and that the same level of activation can be reached by a sequence of interrupted contacts or with one stable contact [62]. The intracellular events

underlying these phenomena are not yet fully elucidated. Accumulation of transcription factors such as cJun over time following suboptimal TCR ligation has been demonstrated [63]; it was postulated that a counting molecule exists allowing for a stepwise buildup of activated signaling intermediates finally leading to T cell activation [63]. The question, whether such a system works like a capacitor, that “deloads” in one stroke, once it has reached the loading capacity or whether a more analog system exists, that can generate a smooth response reflecting different levels of activation is currently only a matter of speculation.

3.4 A serial encounter model of T cell activation

These findings were put together in the serial encounter model for T cell activation [64, 65]. Based on the findings in 3D collagen matrices the serial encounter model stated that there is no difference in the biophysical dynamics of T cells contacting low affinity or cognate APC. However, only cognate APC were able to deliver a signal to the T cell. Thereby, the number of available pMHC complexes on one cell would not be essential, as it has been shown, that even one pMHC molecule is sufficient to trigger T cell responses [64a]. Since one contact was not enough, the T cell was forced to undergo additional contacts to other APC presenting the same antigen. Such a mechanism would be helpful in “measuring” the amount of antigen presenting APC in the migratory reach of a T cell and thereby inhibit unnecessary immune activation in the case of minor infections with very low numbers of antigen specific APC [64].

3.5 The road to intravital imaging – phase models of T cell activation

The results on T cell biophysics obtained in 3D collagen matrices were challenged mainly by the fact that in true lymphatic tissue collagen fibers are not directly accessible to migrating cells. Rather, they are ensheathed by follicular reticulum cells of the lymphatic environment

[26, 66]. Thus, the behavior of T cells in 3D collagen might not necessarily represent the in vivo reality [67-69].

The goal to observe T cell activation in true lymphatic tissue was finally reached by two groups working with naïve T cells and DC in explanted lymph nodes [70, 71] and by a group examining thymocytes in reaggregate thymic organ cultures [6]. All three studies were able to demonstrate effective migration of T cells in lymphatic tissue. This was very remarkable given the extreme crowding of cells in these organs [27]. In fact, for T and B cells, the measured motility parameters in intact lymphatic organs were identical to the values that were obtained for cells migrating in artificial 3D systems, which are only sparsely populated such that individual cells only rarely touch each other [34, 72-74]. Within T cell zones of lymph nodes dendritic cells, in contrast, were found to be more static. Rather than migrating within lymph nodes, they remained mainly stationary, but displayed highly motile dendrites which were constantly reaching for T cells [49, 75]. Interestingly, the studies arrived at opposite conclusions concerning the duration of antigen-specific contacts between T cells and DC. While the studies by Bousso et al. [6], and Miller et al. [70] showed that contacts between T cells and APC were an equal mixture of short-lived and more extended interactions, Stoll et al. found exclusively long-lasting contacts between adoptively transferred mature DC and naïve T cells upon antigen presentation [71]. It was possible that the differential results were due to differences in treatment of explanted tissues or that changes in the behavior of cells after removal of the lymph nodes occurred. Therefore, it was clear that it was necessary to approach imaging in lymph nodes in situ, i.e. in the living animal.

This goal was initially reached by Miller et al., albeit only by looking at T cell migration [76]. The first intravital study of naïve T cells interacting with DC in a lymph node was completed by Mempel et al. [28]. Monitoring CD8⁺ T cells this study showed that the T cell activation followed a three step mechanism. T cells which had just entered the lymph node engaged antigen-specific DC in short, transient encounters during the first 6-8 hours. After this time

point interactions became more stable (in the range of one or more hours) and the production of effector cytokines was induced. This phase lasted for ~ 12 h. A third stage was again characterized by profound motility and only transient encounters with DC. It was during this phase that T cell divisions became detectable [28]. These findings were essentially confirmed for CD4⁺ T cell activation in vivo [52] and in an explanted lymph node model [50]. In addition, the group of Amigorena showed that CD8⁺ T cells contacting DC under a regime inducing tolerance rather than activation displayed short-lived interactions at any time point, while Mempel's three phase model with long interactions in a middle phase [28] could be confirmed for an activatory situation [77]. Thus, current data suggest, that a complete absence of static interactions to DC is associated with less efficient T cell activation or even T cell tolerization for CD8 cells, while there seems to be no obvious difference of interaction behavior of CD4 T cells, whether the outcome is anergy or activation [52]. In any case, most available studies clearly show the presence of dynamic interactions at different time points during effective T cell stimulation.

The exact function and underlying molecular mechanisms of each respective phase are unclear, however, the sequence may reflect expression changes of surface receptors and cytoskeletal regulators during T cell activation. It is possible that the longevity of T-DC interactions in a phase might indicate the formation of a mature synapse-like contact zone [78]. While both dynamic and stable contacts contribute to T cell activation and proliferation, long-lasting interactions may be needed for maximum IL-2 production and gaining of full effector functions [79]. The phase model or kinetic transition model of T cell interaction expands the serial encounter model by postulating an integration of several signals of different duration and strengths into a common pathway of T cell activation.

3.6 The role of the APC

The results of the recent studies lead to another important question: What cell makes the decision on maintaining the interaction and co-migration? The T cell or the APC? The studies by Mempel [28] and Miller [50] concluded that it was the status of the T cell which decided about the duration of APC interaction. However, even in the rather homogeneous population of naïve TCR transgenic T cells, interactions with DC were shown to differ substantially in vitro, ranging from short-lived to long-lived [79]. The first study addressing the role of the APC for deciding on the duration of interaction used two types of effective APC, DC and activated B cells, as well as naïve B cells as a model for a weak, ineffective APC. The APC were peptide-loaded and incubated separately with naïve TCR transgenic T cells. It was found that the T cells contacted effective APC, DC and activated B cells, in a short-lived manner, while they formed long-lasting contacts with inefficient APC, naïve B cells. This pattern was demonstrated both in 3D in vitro systems and in lymph nodes in vivo [34]. The findings were even more unexpected as the long-lasting contacts with naïve B cells were shown to be the least efficient in terms of T cell activation, while the much more dynamic contacts to DC and activated B cells rapidly induced T cell activation [34].

3.7 Summary: A multitude of T-APC interaction kinetics

For the original problem of how to synchronize the migration of cells initially separated in space, live cell imaging experiments of the past five years have offered a wide range of solutions. Synchronization can occur transiently by limiting the migration of T cells to the surface of APC, either with an almost unchanged velocity compared to free migration or in a more stable manner with strongly reduced motility down to complete stopping of T cells on the surface of DC. In a most extreme form, hour-long coordinated migration of a cell pair occurs, as in the example of naïve B cells as APC [34]. During the co-migration, very motile T cells were shown to push B cells in vivo through lymph node parenchyma, whereas B cells, when activated, may take the lead themselves and pull T cells [34]. The latter finding was later confirmed for B cells which were activated by loading antigen onto their B cell receptor [32]. Another issue covered by the studies mentioned was directional migration. In the marketplace analogy, this would mean that customers on the market would somehow be preferentially attracted to or by specific booths. Currently available data do not support this idea. In contrast, there is evidence that migration of T cells in lymph nodes is entirely random and that encounters with APC are stochastic events [76, 80]. However, other researchers call these conclusions into question [81]. What is agreed upon is the sole fact that the lymph node

per se is attractive to T cells, B cells and APC by means of the chemokines CCL19 and CCL21 binding to the chemokine receptor CCR7 [82].

Lastly, it was shown that the character of interaction is not only modulated by antigenic load and type of APC, but the architecture of the surrounding tissue may influence the dynamics of cell interactions, too. Tight dendritic cell networks within the lymph node facilitate in vivo T cell contacts with DC and the transition between them, allowing the T cells to be in contact with DC for most of the time [75, 83].

4. Conclusions and Outlook

Great advantages in imaging techniques in recent years have led to unique insights into the complexity of cell-cell interactions in vivo. However, the resulting models of T cell activation still vary in their interpretation of physical interaction modes of T cells and many aspects remain yet descriptive.

There is now evidence from several research groups for a multi-step cell interaction process during T cell activation. However, the molecular basis for the different interaction kinetics observed remains unclear. Other questions in need of clarification are e.g.: What is the relative importance of long over short contacts for T cell activation? Are both contact types necessary or is one dispensable in vivo? By what molecular principle do short cell contacts differ from long ones? Do short contacts “prepare” cells for long ones and if yes, how? How exactly is the surrounding tissue influencing the duration or outcome of interaction? Apart from studying effector cell induction, the mechanism of APC activation (especially of B cells) needs to be investigated. Experimental models need to be expanded to include locations other than inguinal or popliteal lymph nodes, e.g. lymphatic sites in the gut and the lung. More physiologically relevant models together with pathogen-related antigens are needed as well as ways to overcome one major obstacle for in vivo imaging studies, the low number of precursor cells in wild type animals.

In conclusion, T cells display an intriguingly complex pattern of cellular interaction with APC during their activation. A more complete understanding of the underlying mechanisms in vivo is needed to modulate or mimic such processes. This would be one prerequisite to make them amenable for therapeutic intervention. Based on the excellent advances in intravital imaging made in recent years we can be very positive about future insights gained from this powerful methodology.

Acknowledgments:

We thank Priyanka Narang for critical reading of the manuscript.

References:

1. Friedl, P. and E.B. Brocker, *TCR triggering on the move: diversity of T-cell interactions with antigen-presenting cells*. Immunol Rev, 2002. **186**: p. 83-9.
2. Nikolich-Zugich, J., M.K. Slifka, and I. Messaoudi, *The many important facets of T-cell repertoire diversity*. Nat Rev Immunol, 2004. **4**(2): p. 123-32.
3. Goldrath, A.W. and M.J. Bevan, *Selecting and maintaining a diverse T-cell repertoire*. Nature, 1999. **402**(6759): p. 255-262.
4. Sebzda, E., et al., *SELECTION OF THE T CELL REPERTOIRE*. Annu. Rev. Immunol., 1999. **17**(1): p. 829-874.
5. Correia-Neves, M., et al., *The shaping of the T cell repertoire*. Immunity, 2001. **14**(1): p. 21-32.
6. Bousso, P., et al., *Dynamics of thymocyte-stromal cell interactions visualized by two-photon microscopy*. Science, 2002. **296**(5574): p. 1876-80.
7. Richie, L.I., et al., *Imaging synapse formation during thymocyte selection: inability of CD3zeta to form a stable central accumulation during negative selection*. Immunity, 2002. **16**(4): p. 595-606.
8. Alberola-Ila, J., et al., *Selective requirement for MAP kinase activation in thymocyte differentiation*. Nature, 1995. **373**(6515): p. 620-3.
9. Bhakta, N.R., D.Y. Oh, and R.S. Lewis, *Calcium oscillations regulate thymocyte motility during positive selection in the three-dimensional thymic environment*. Nat Immunol, 2005. **6**(2): p. 143-51.
10. Yasutomo, K., et al., *The duration of antigen receptor signalling determines CD4+ versus CD8+ T-cell lineage fate*. Nature, 2000. **404**(6777): p. 506-10.
11. Witt, C.M., et al., *Directed migration of positively selected thymocytes visualized in real time*. PLoS Biol, 2005. **3**(6): p. e160.
12. Palmer, E., *Negative selection--clearing out the bad apples from the T-cell repertoire*. Nat Rev Immunol, 2003. **3**(5): p. 383-91.
13. Butcher, E.C. and L.J. Picker, *Lymphocyte homing and homeostasis*. Science, 1996. **272**(5258): p. 60-6.
- 13a. Gunzer, M., et al., *Systemic administration of a TLR7 ligand leads to transient immune incompetence due to peripheral blood leukocyte depletion*. Blood, 2005.(in Press)
14. Potsch, C., D. Vohringer, and H. Pircher, *Distinct migration patterns of naive and effector CD8 T cells in the spleen: correlation with CCR7 receptor expression and chemokine reactivity*. Eur J Immunol, 1999. **29**(11): p. 3562-70.
15. Moser, B. and P. Loetscher, *Lymphocyte traffic control by chemokines*. Nat Immunol, 2001. **2**(2): p. 123-8.
16. Mackay, C.R., *Homing of naive, memory and effector lymphocytes*. Curr Opin Immunol, 1993. **5**(3): p. 423-7.
17. Bradley, L.M. and S.R. Watson, *Lymphocyte migration into tissue: the paradigm derived from CD4 subsets*. Curr Opin Immunol, 1996. **8**(3): p. 312-20.
- 17a. Brocker, T., *Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class-II expressing dendritic cells*. J.Exp.Med., 1997. **186** (8): p. 1223-1232.
18. Itano, A.A., et al., *Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity*. Immunity, 2003. **19**(1): p. 47-57.
19. Cella, M., et al., *Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells*. Nature, 1997. **388**(6644): p. 782-7.

20. Kedl, R.M., et al., *T cells compete for access to antigen-bearing antigen-presenting cells*. J Exp Med, 2000. **192**(8): p. 1105-13.
21. Mellman, I. and R.M. Steinman, *Dendritic cells: specialized and regulated antigen processing machines*. Cell, 2001. **106**(3): p. 255-8.
22. Romani, N., et al., *Migration of dendritic cells into lymphatics-the Langerhans cell example: routes, regulation, and relevance*. Int Rev Cytol, 2001. **207**: p. 237-70.
23. Lukas, M., et al., *Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model*. J Invest Dermatol, 1996. **106**(6): p. 1293-9.
24. Mehling, A., et al., *Overexpression of CD40 ligand in murine epidermis results in chronic skin inflammation and systemic autoimmunity*. J Exp Med, 2001. **194**(5): p. 615-28.
25. Gretz, J.E., A.O. Anderson, and S. Shaw, *Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex*. Immunol Rev, 1997. **156**: p. 11-24.
26. Gretz, J.E., et al., *Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex*. J Exp Med, 2000. **192**(10): p. 1425-40.
27. Ushiki, T., O. Ohtani, and K. Abe, *Scanning electron microscopic studies of reticular framework in the rat mesenteric lymph node*. Anat Rec, 1995. **241**(1): p. 113-22.
28. Mempel, T.R., S.E. Henrickson, and U.H. Von Andrian, *T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases*. Nature, 2004. **427**(6970): p. 154-9.
29. Bajenoff, M., S. Granjeaud, and S. Guerder, *The strategy of T cell antigen-presenting cell encounter in antigen-draining lymph nodes revealed by imaging of initial T cell activation*. J Exp Med, 2003. **198**(5): p. 715-24.
30. Fuchs, E.J. and P. Matzinger, *B cells turn off virgin but not memory T cells*. Science, 1992. **258**(5085): p. 1156-9.
31. Rodriguez-Pinto, D. and J. Moreno, *B cells can prime naive CD4+ T cells in vivo in the absence of other professional antigen-presenting cells in a CD154-CD40-dependent manner*. Eur J Immunol, 2005. **35**(4): p. 1097-105.
32. Okada, T., et al., *Antigen-Engaged B Cells Undergo Chemotaxis toward the T Zone and Form Motile Conjugates with Helper T Cells*. PLoS Biol, 2005. **3**(6): p. e150.
33. Reif, K. et al. *Balanced responsiveness to chemoattractants from adjacent zones determines B cell position*. Nature, 2002. **416**: p. 94-9.
34. Gunzer, M., et al., *A spectrum of biophysical interaction modes between T cells and different antigen-presenting cells during priming in 3-D collagen and in vivo*. Blood, 2004. **104**(9): p. 2801-9.
35. Kannagi, R., *Regulatory roles of carbohydrate ligands for selectins in the homing of lymphocytes*. Curr Opin Struct Biol, 2002. **12**(5): p. 599-608.
36. Springer, T.A., *Adhesion receptors of the immune system*. Nature, 1990. **346**(6283): p. 425-34.
37. Springer, T.A., *Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm*. Cell, 1994. **76**(2): p. 301-14.
38. Hwang, J.M., et al., *A critical temporal window for selectin-dependent CD4+ lymphocyte homing and initiation of late-phase inflammation in contact sensitivity*. J Exp Med, 2004. **199**(9): p. 1223-34.
39. Sallusto, F., J. Geginat, and A. Lanzavecchia, *Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance*. Annu. Rev. Immunol., 2004. **22**(1): p. 745-763.

40. Seder, R.A. and R. Ahmed, *Similarities and differences in CD4+ and CD8+ effector and memory T cell generation*. Nat. Immunol., 2003. **4**(9): p. 835-842.
41. Sallusto, F., et al., *Two subsets of memory T lymphocytes with distinct homing potentials and effector functions*. Nature, 1999. **401**(6754): p. 708-12.
42. Jonuleit, H., et al., *Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood*. J Exp Med, 2001. **193**(11): p. 1285-94.
43. Probst, H.C., et al., *Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance*. Immunity, 2003. **18**(5): p. 713-20.
44. O'Neill, E.J., et al., *Natural and Induced Regulatory T Cells*. Ann NY Acad Sci, 2004. **1029**(1): p. 180-192.
45. Sakaguchi, S., *Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self*. Nat Immunol, 2005. **6**(4): p. 345-52.
46. Fontenot, J.D., et al., *Regulatory T cell lineage specification by the forkhead transcription factor foxp3*. Immunity, 2005. **22**(3): p. 329-41.
47. Negulescu, P.A., et al., *Polarity of T cell shape, motility, and sensitivity to antigen*. Immunity., 1996. **4**(5): p. 421-430.
48. Valitutti, S., et al., *Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton*. J.Exp.Med., 1995. **181**: p. 577-584.
49. Miller, M.J., et al., *T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node*. Proc Natl Acad Sci U S A, 2004. **101**(4): p. 998-1003.
50. Miller, M.J., et al., *Imaging the single cell dynamics of CD4+ T cell activation by dendritic cells in lymph nodes*. J Exp Med, 2004. **200**(7): p. 847-56.
51. Lee, K.H., et al., *The immunological synapse balances T cell receptor signaling and degradation*. Science, 2003. **302**(5648): p. 1218-22.
52. Shakhar, G., et al., *Stable T cell-dendritic cell interactions precede the development of both tolerance and immunity in vivo*. Nat Immunol, 2005, **6** (7): p. 707-714
53. Gunzer, M., et al., *Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential*. Immunity, 2000. **13**(3): p. 323-32.
54. Kupfer, A. and S.J. Singer, *The specific interaction of helper T cells and antigen-presenting B cells. IV. Membrane and cytoskeletal reorganizations in the bound T cell as a function of antigen dose*. J Exp Med, 1989. **170**(5): p. 1697-713.
55. Monks, C.R., et al., *Three-dimensional segregation of supramolecular activation clusters in T cells*. Nature, 1998. **395**(6697): p. 82-6.
56. Iezzi, G., K. Karjalainen, and A. Lanzavecchia, *The duration of antigenic stimulation determines the fate of naive and effector T cells*. Immunity, 1998. **8**(1): p. 89-95.
57. Dustin, M.L., et al., *Antigen receptor engagement delivers a stop signal to migrating T lymphocytes*. Proc Natl Acad Sci U S A, 1997. **94**(8): p. 3909-13.
58. Dustin, M.L. and A.C. Chan, *Signaling takes shape in the immune system*. Cell, 2000. **103**(2): p. 283-94.
59. Lanzavecchia, A., G. Lezzi, and A. Viola, *From TCR engagement to T cell activation: a kinetic view of T cell behavior*. Cell, 1999. **96**(1): p. 1-4.
60. Underhill, D.M., et al., *Dynamic interactions of macrophages with T cells during antigen presentation*. J Exp Med, 1999. **190**(12): p. 1909-14.
61. Borovsky, Z., et al., *Serial triggering of T cell receptors results in incremental accumulation of signaling intermediates*. J Biol Chem, 2002. **277**(24): p. 21529-36.
62. Faroudi, M., et al., *Cutting edge: T lymphocyte activation by repeated immunological synapse formation and intermittent signaling*. J Immunol, 2003. **171**(3): p. 1128-32.

63. Rosette, C., et al., *The impact of duration versus extent of TCR occupancy on T cell activation: a revision of the kinetic proofreading model*. Immunity, 2001. **15**(1): p. 59-70.
64. Friedl, P. and M. Gunzer, *Interaction of T cells with APCs: the serial encounter model*. Trends Immunol, 2001. **22**(4): p. 187-91.
- 64a. D. J. Irvine, M. A. Purbhoo, M. Krogsgaard, and M. M. Davis. Direct observation of ligand recognition by T cells. *Nature* 419 (6909):845-849, 2002.
65. Rachmilewitz, J. and A. Lanzavecchia, *A temporal and spatial summation model for T-cell activation: signal integration and antigen decoding*. Trends Immunol, 2002. **23**(12): p. 592-5.
66. Kaldjian, E.P., et al., *Spatial and molecular organization of lymph node T cell cortex: a labyrinthine cavity bounded by an epithelium-like monolayer of fibroblastic reticular cells anchored to basement membrane-like extracellular matrix*. Int Immunol, 2001. **13**(10): p. 1243-53.
67. Dustin, M.L. and A.R. de Fougères, *Reprogramming T cells: the role of extracellular matrix in coordination of T cell activation and migration*. Curr Opin Immunol, 2001. **13**(3): p. 286-90.
68. Dustin, M.L., P.M. Allen, and A.S. Shaw, *Environmental control of immunological synapse formation and duration*. Trends Immunol, 2001. **22**(4): p. 192-4.
69. Lanzavecchia, A. and F. Sallusto, *Antigen decoding by T lymphocytes: from synapses to fate determination*. Nat Immunol, 2001. **2**(6): p. 487-92.
70. Miller, M.J., et al., *Two-photon imaging of lymphocyte motility and antigen response in intact lymph node*. Science, 2002. **296**(5574): p. 1869-73.
71. Stoll, S. Delon, J., Brotz, T. M. and R. N. Germain. Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science*, 2002, **296**(5574): p. 1873-1876.
72. Friedl, P., et al., *CD4+ T lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize beta1 integrin-independent strategies for polarization, interaction with collagen fibers and locomotion*. Eur J Immunol, 1998. **28**(8): p. 2331-43.
73. Friedl, P., P.B. Noble, and K.S. Zanker, *T lymphocyte locomotion in a three-dimensional collagen matrix. Expression and function of cell adhesion molecules*. J Immunol, 1995. **154**(10): p. 4973-85.
74. Friedl, P., et al., *Locomotor phenotypes of unstimulated CD45RA^{high} and CD45RO^{high} CD4⁺ and CD8⁺ lymphocytes in three-dimensional collagen lattices*. Immunology, 1994. **82**(4): p. 617-24.
75. Lindquist, R.L., et al., *Visualizing dendritic cell networks in vivo*. Nat Immunol, 2004. **5**(12): p. 1243-50.
76. Miller, M.J., et al., *Autonomous T cell trafficking examined in vivo with intravital two-photon microscopy*. Proc Natl Acad Sci U S A, 2003. **100**(5): p. 2604-9.
77. Hugues, S., et al., *Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity*. Nat Immunol, 2004. **5**(12): p. 1235-42.
78. Lee, K.H., et al., *T cell receptor signaling precedes immunological synapse formation*. Science, 2002. **295**(5559): p. 1539-42.
79. Hurez, V., et al., *Restricted clonal expression of IL-2 by naive T cells reflects differential dynamic interactions with dendritic cells*. J Exp Med, 2003. **198**(1): p. 123-32.
80. Wei, S.H., et al., *A stochastic view of lymphocyte motility and trafficking within the lymph node*. Immunol Rev, 2003. **195**: p. 136-59.
81. Germain, R.N. and M.K. Jenkins, *In vivo antigen presentation*. Curr Opin Immunol, 2004. **16**(1): p. 120-5.

82. Forster, R., et al., *CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs*. Cell, 1999. **99**(1): p. 23-33.
83. Westermann, J., et al., *Naive, effector, and memory T lymphocytes efficiently scan dendritic cells in vivo: contact frequency in T cell zones of secondary lymphoid organs does not depend on LFA-1 expression and facilitates survival of effector T cells*. J Immunol, 2005. **174**(5): p. 2517-24.