T-cell activation by dendritic cells in the lymph node: lessons from the movies

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Abstract | Interactions between T cells and dendritic cells (DCs) in the lymph nodes are crucial for initiating cell-mediated adaptive immune responses. With the help of two-photon imaging, the complexity of these cellular contacts *in vivo* has recently been captured in time-lapse movies in several immunological contexts. Well beyond the satisfaction of seeing a T-cell response as it happens, these experiments provide fundamental insights into the regulation and the biological meaning of T-cell–DC contact dynamics. This Review focuses on how this emerging field is changing our perception of T-cell activation by DCs.

Lymph nodes
Secondary lymphoid organs that collect cells from the blood and the afferent lymph, and antigens from the lymph for presentation to T and B cells. The human body contains several hundred lymph nodes.

Two-photon laser scanning microscopy
(TPLSM). Laser-scanning microscopy that uses pulsed infrared laser light for the excitation of conventional fluorophores or fluorescent proteins. This technique greatly reduces photodamage of living specimens and improves depth of tissue penetration, owing to the low level of light scattering within the tissue.

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doi:10.1038/nri2379
Published online
8 August 2008

The lymph nodes are sites where naive T cells that are in search of their cognate antigen have the opportunity to survey dendritic cells (DCs), the most potent antigen-presenting cells (APCs), for their activation[1]. The establishment of a cellular contact between a T cell and a DC provides the opportunity for antigen recognition to occur through T-cell receptor (TCR) interactions with peptide–MHC complexes that are present at the DC surface. In addition, T-cell–DC contacts involve various receptor–ligand interactions (including those of co-stimulatory and adhesion molecules) and the delivery of soluble mediators that modulate the outcome of T-cell activation. Productive activation of naive T cells by DCs results in their clonal expansion and differentiation into effector and memory T cells.

Despite our fundamental understanding of T-cell activation, the way individual T cells experience their encounters with DCs *in vivo* has remained largely unknown. A naive T cell spends an average of 24 hours in a given lymph node, but this duration is extended to 3–4 days when the T cell is exposed to its cognate antigen. What precisely happens during this period has long been considered a black box, as many important questions remain unanswered. For example, what are the mechanisms that favour productive T-cell–DC encounters? How is the sequence of cellular events that lead to T-cell activation regulated? How do T cells collect and integrate signals during their interactions with DCs? Solving these pieces of the puzzle is fundamental, as these factors have an integral role in shaping T-cell responses.

Since 2002, the answer to some of these questions has come in the form of time-lapse movies generated by two-photon laser scanning microscopy (TPLSM), now considered the technique of choice to 'spy' on the interaction between T cells and DCs in secondary lymphoid organs[2,3]. Real-time imaging of T-cell–DC interactions in lymph nodes delivers new information that has brought new questions to our attention. This article reviews our current understanding of *in vivo* T-cell–DC contact dynamics, emphasizing their multiple modes of regulation and their contribution to the outcome of T-cell activation.

Under the eye of two-photon microscopy

In lymph nodes, most T cells are located in the paracortical area, which in the mouse is found approximately 150–200 μm below the organ's surface. With the ability to image fluorescently labelled cells deep in the tissues while minimizing photoxicity, TPLSM is perfectly suited for the visualization of the dynamics of T-cell activation in a native environment at single-cell resolution[4]. For a detailed description of TPLSM and its application for immunoimaging, I refer the reader to some excellent reviews[5,6].

Visualization of T-cell–DC interactions can be carried out in explanted lymph nodes or by using an intravital preparation in anesthetized mice (BOX 1, FIG. 1a). Practically, a two-photon imaging experiment is carried out by acquiring sequential images of a three-dimensional volume of a lymph node that contains fluorescently labelled T cells and DCs. This is achieved by recording the fluorescence signals at successive focal planes and repeating this process every 10–30 seconds

Box 1 | Lymph-node explants and intravital preparations

Cahalan and colleagues carried out the first two-photon study of $CD4^+$ T-cell activation[3]. They used explanted inguinal lymph nodes that were maintained at 37°C and perfused with media bubbled with a gas mixture containing 95% O_2 and 5% CO_2. In these settings, which resemble those used by neurobiologists to image brain slices, both a physiological temperature and the presence of oxygen were crucial for observing vigorous T-cell motility. A refined analysis of the perfusion requirement for the proper imaging of lymph-node explants has been provided recently[61]. Subsequently, preparations for intravital imaging of the inguinal and the popliteal lymph nodes were described and confirmed the motile behaviour of T cells that has been seen in explants[35,62,63]. Although intravital preparations have the advantage of maintaining blood and lymph flow, they are more time-consuming, which makes it difficult to image multiple lymph nodes in the same experiment. One interesting advantage that is conferred by intravital imaging is the possibility of injecting a molecule of interest into the blood stream and watching its effect in real-time as it diffuses in secondary lymphoid organs. This approach was used successfully to visualize changes in T-cell–DC contacts following intravenous injection of antigen[22,45], of an MHC class II-specific antibody[45] or changes in T-cell motility following subcutaneous injection of the CC-chemokine ligand 19 (CCL19)[64].

for periods of up to a few hours (FIG. 1b). The multi-dimensional data set that is generated can be displayed as a time-lapse movie (see Supplementary information S1 (movie)), and important information can then be extracted from these videos through manual or automated analyses (FIG. 1c). For example, measurement of T-cell velocity and trajectory confinement (that is, whether T cells move in a confined area) can be used to track the efficiency of T-cell arrest during antigen recognition. In addition, counting the rate of T-cell–DC encounters can offer insights into the contribution of chemokine-driven migration in this process. Individual contacts that are followed over time provide information on the stability of T-cell–DC interactions. Furthermore, real-time monitoring of cytosolic Ca^{2+} concentration in T cells using TPLSM approaches might help to identify when and where signals are exchanged. Undoubtedly, read-outs that detect signalling molecules or that assess gene expression will soon be included with imaging experiments to further extend the biological questions that can be addressed with this methodology. Similar to other assays, the most meaningful measurements are obtained in studies that compare different experimental conditions: for example, when mutant and wild-type T cells or DCs are compared, or when investigating different immunization regimens. Last, some of the measurements derived from two-photon movies can be used to calibrate computational models to test specific hypotheses and make new predictions about T-cell migration and activation[7,8].

Studying T-cell–DC dynamics *in vivo*

Visualizing T cells. T cells in lymph nodes migrate at high velocities, on average at about 11–14 μm per minute[3]. Their trajectories are largely chaotic, if not random. However, a closer look at their intranodal migration revealed a role for the fibroblastic reticular-cell network in guiding T-cell movement[9]. To track T cells, most experiments have relied on the adoptive transfer of high numbers of fluorescently tagged $CD4^+$ or $CD8^+$ T cells that express a transgenic TCR. With this strategy, it is possible to introduce large numbers of T cells so that many of them can be detected in the lymph node that is imaged. Furthermore, it ensures that all imaged T cells have a common TCR specificity and display relatively homogenous behaviour in response to antigen.

However, this methodology is not without potential drawbacks. In particular, a high T-cell precursor frequency increases the occurrence of intraclonal T-cell competition and affects the outcome of T-cell activation, especially if the antigen is limiting[10,11]. In this respect, profound differences in the dynamics of T-cell–DC interactions have been observed by varying the number of adoptively transferred T cells[12]. Stable T-cell–DC interactions were rapidly inhibited when the number of responding T cells was too high, thereby providing a cellular basis for intraclonal competition and suggesting the existence of a feedback mechanism for the termination of T-cell clonal expansion. An important implication of this observation is that care should be taken when interpreting T-cell–DC contact dynamics in experimental settings that favour T-cell competition.

Visualizing dendritic cells. Visualization of DCs has been achieved using a number of different approaches[13]. The first strategy relies on the intradermal or subcutaneous injection of a purified DC population[14,15]. Bone-marrow-derived or splenic DCs that are labelled with a vital dye or that express a fluorescent protein can be pulsed with MHC class I- or MHC class II-restricted peptides at the desired concentration. DCs can also be pulsed simultaneously with two distinct antigenic peptides and exposed to mixed populations of T cells to study the occurrence of interactions between three cells[12,16,17]. Approximately 12–18 hours post-injection, a fraction of the transferred DCs is found in the draining lymph nodes and typically expresses high levels of MHC class II and co-stimulatory molecules. In the lymph nodes, these migratory DCs are slow motile cells (and move at a rate of 3–6 μm per minute) that display frequent shape changes and dendrite extensions (see Supplementary information S2 (movie)). Within 2 days, these DCs lose their motility as they become integrated into the network of lymph-node DCs and die rapidly.

The second approach relies on a mouse strain that was generated by Nussenzweig and colleagues in which the yellow fluorescent protein molecule is expressed under the control of the CD11c promoter[18]. In these mice, the strong fluorescent signal readily reveals a network of lymph-node resident DCs that is made of tightly packed sessile cells (see Supplementary information S2 (movie)). This model is particularly useful for the study of T-cell interactions with endogenous lymph-node DCs following the delivery of antigen. Because both antigen-bearing and non-bearing DCs are fluorescent, strategies that deliver antigen to a large fraction of lymph-node DCs are preferred so that it can be assumed that most DCs have the capability to establish cognate interactions with T cells. For example, coupling antigen to an antibody that is specific for the endocytic receptor DEC-205 has been used by several groups to target antigen to DEC-205^+ DCs[19,20].

Phototoxicity
The phenomenon by which illumination of fluorescent molecules in a cell causes damage and eventually cell death, most likely owing to the formation of oxygen radicals.

Intraclonal T-cell competition
The process during which numerous T cells face a limiting resource (such as antigen or cytokines) that results in a diminished T-cell activation efficiency on a per cell basis.

DEC-205
A membrane glycoprotein expressed by $CD8^+$ DCs that acts as an endocytic receptor. Genetic or chemical coupling of antigenic fragments to a DEC-205-specific antibody permits efficient delivery of the conjugate to a large proportion of DCs in lymphoid tissues.

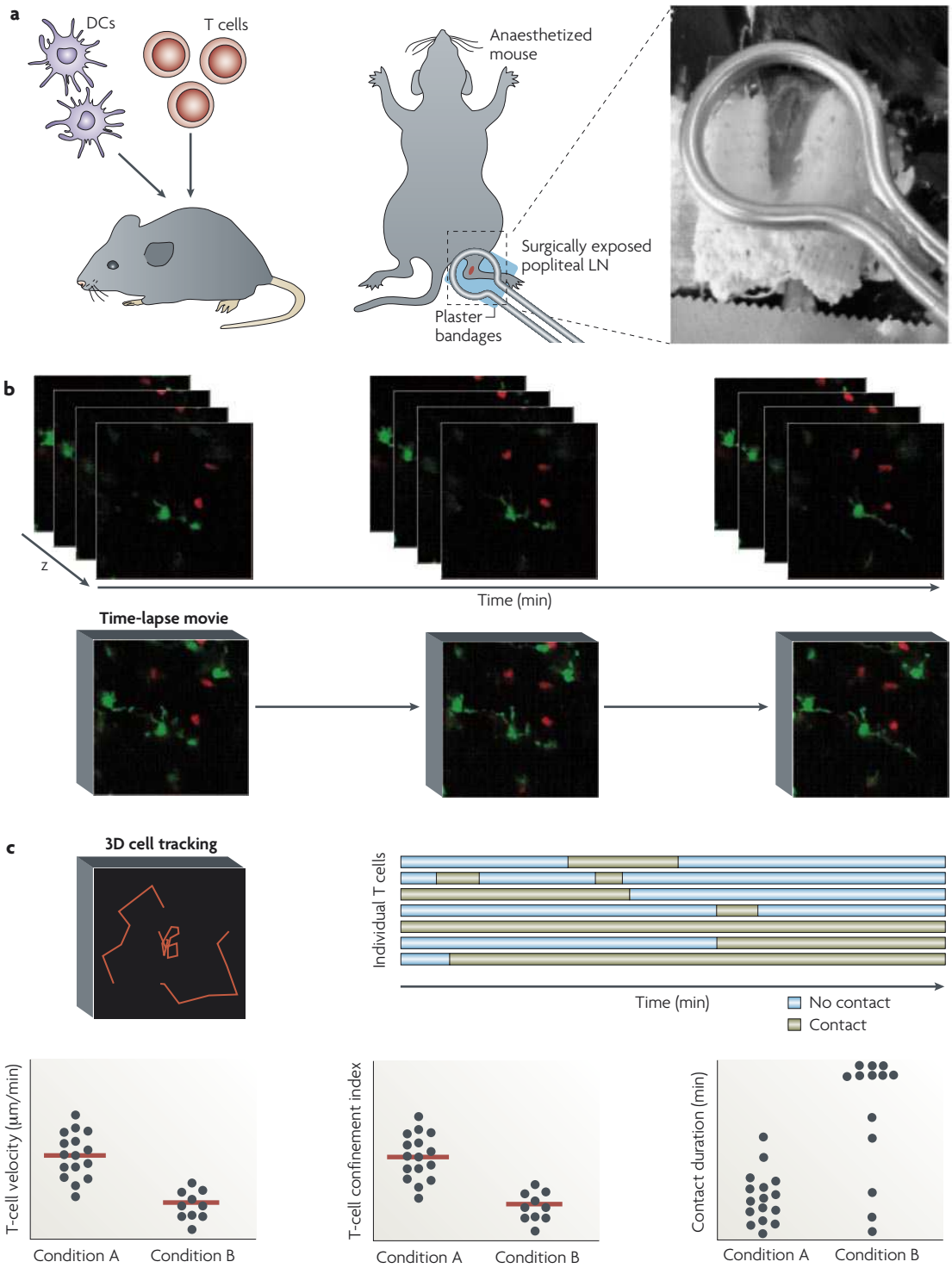

Figure 1 | **Intravital two-photon imaging of T-cell–DC interactions. a** | The popliteal lymph node of an anesthetized mouse that contains fluorescently labelled T cells and dendritic cells (DCs) is surgically exposed. The mouse leg is immobilized with plaster bandages and a heated metal ring is placed around the sample to maintain it at physiological temperature. The animal is then placed under an upright two-photon microscope (for more details, see REF. 63). **b** | For image acquisition, the area of interest is scanned by a near infrared-pulsed laser to generate *x*–*y* images. The process is carried out at different *z* planes and repeated every 10–30 seconds. The multidimensional data set is then used to generate a time-lapse movie. **c** | For data extraction, mean cell velocities and confinements are obtained and graphed for individual T cells after automated 3D cell tracking. In addition, DC interactions that are experienced by individual T cells can be monitored over time. These data can be used, for example, to generate graphs that compile information regarding T-cell–DC contact durations. Part **a** (right panel) modified, with permission, from REF. 63 © (2007) Springer Science + Business Media.

Finally, in a third approach, subcutaneous injection of the CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester) fluorescent dye together with cytokines and antigen that is adsorbed to the adjuvant alum has been used to deliver antigen to and label endogenous skin DCs that subsequently traffic to the lymph nodes[21]. It should be noted that each of these approaches targets subsets of DCs that might differ in their ability to interact with and activate antigen-specific naive T cells.

Visualizing the antigen. In addition to methods for tracking DCs, alternative approaches have been developed to visualize the presence of antigen within APCs. For example, an autoquenched version of the ovalbumin protein (OVA) that becomes fluorescent on proteolytic cleavage inside APCs can be injected intravenously to visualize the kinetics of antigen processing and presentation to T cells[22]. In addition, Tang *et al.* used a mouse strain in which green fluorescent protein (GFP) is expressed under the control of the mouse insulin 1 promoter on the non-obese diabetic (NOD)-genetic background to track endogenous DCs that had engulfed GFP-positive cells[23]. They noted the presence of 20–300 GFP-positive DCs in the pancreatic lymph nodes of these mice and showed that these cells were actively presenting islet antigens to both $CD4^+CD25^-$ T-helper cells and regulatory T cells. Finally, recombinant viruses expressing GFP molecules that were fused to a model antigenic peptide and the influenza nucleoprotein have been used to identify virus-infected cells and to analyse their interactions with antigen-specific $CD8^+$ T cells[24].

Diversity of T-cell–DC contacts *in vivo*

Results of *in vitro* experiments support the idea that antigen recognition by T cells is associated with T-cell arrest on the APC (also referred to as the T-cell stop signal) and the formation of long-lasting contacts[25–27]. This view was challenged by the finding that naive T cells established multiple short contacts (less than 10 minutes in duration) with antigen-bearing DCs when placed in a collagen matrix, and became activated by this process[28]. Two-photon imaging of T-cell–DC contacts *in vivo* was an ideal way to resolve this controversy and to determine whether T-cell activation *in vivo* relies on interactions with DCs that last for minutes or hours. However, instead of providing the simple answer researchers were looking for, experiments carried out by several groups in different experimental systems revealed that there is a high degree of diversity in T-cell–DC contact dynamics. This complexity raised the exciting hypothesis that the regulation of T-cell–DC contact dynamics was an essential component of the T-cell activation process.

Contacts in the absence of antigen. Owing to the vigorous intranodal motility of T cells, individual DCs in the lymph node constantly experience new encounters with T cells (see Supplementary information S2 (movie)). Imaging of T-cell–DC contacts in the absence of antigen revealed that 500–5,000 distinct T cells were 'seen' by each DC every hour[15,21]. Such impressive rates of encounter provided a satisfactory explanation for how a small number of antigen-bearing DCs can establish a 'dialogue' with rare antigen-specific T cells to initiate an immune response. During an encounter with a DC that is not presenting cognate antigen, a T cell does not stop crawling but might experience a slight deceleration and remain in contact for 3–5 minutes. Although calcium signals in T cells during these brief encounters are infrequent[29,30], these contacts might be functionally important, as naive $CD4^+$ T cells that are prevented from engaging MHC class II molecules on the surface of APCs progressively lose their motility in lymph nodes[31].

The rate of T-cell–DC contacts in the absence of antigen recognition is not solely the result of random collision events. $CD8^+$ T cells tend to encounter DCs that have already established a cognate interaction with another $CD4^+$ or a $CD8^+$ T cell more frequently[16,17,32]. Chemokines, such as CC-chemokine ligand 3 (CCL3) and CCL4, are produced at the site of T-cell–DC conjugates and increase the chance for migrating CC-chemokine receptor (CCR5)-expressing $CD8^+$ T cells to contact DCs by a factor of 2 to 4 (REFS 16,32). This mechanism could drive the recruitment of $CD8^+$ T cells by the most competent DCs and favour the formation of three-cell clusters as well as promote collaboration between T cells. Finally, T-cell behaviour can also be influenced by the presence of chemokines that are bound to the surface of DCs. When T cells encounter APCs that have been pulsed with CCR7 ligands, they remain attached to the APCs for several minutes by a uropodal tether, a structure that does not require antigen but results in increased antigen responsiveness of the T cells upon subsequent interactions with APCs[33]. It will be important to determine whether this phenomenon, which is observed *in vitro*, also occurs in intact lymph nodes.

Transient contacts in the presence of antigen. T-cell interactions with antigen-bearing DCs can result in transient, dynamic contacts that last no more than 10 minutes (TABLE 1). In fact, these contacts are hardly distinguishable from those of T cells that are interacting with DCs in the absence of antigen[34,35]. Conceivably, these interactions could be completely unproductive, with T cells simply 'missing' the detection of cognate antigen on the DCs if, for example, limiting amounts of peptide–MHC complexes were present at the contact zone. However, data in the literature suggest that at least a fraction of these interactions results in some level of T-cell activation. For example, following immunization with OVA that is conjugated to a DEC-205-specific antibody (anti-DEC-205–OVA), a regimen that induces an initial state of activation followed by tolerance, the vast majority of OVA-specific $CD8^+$ T cells do not arrest on DCs[36]. This observation indicates that T-cell activation signals can be delivered *in vivo* in the absence of T-cell arrest. A similar conclusion was drawn when studying $CD4^+$ T-cell activation and anergy following administration of a DEC-205-specific antibody that is conjugated to a low potency ligand[37]. The first hours of $CD8^+$ T cells interacting with peptide-pulsed DCs can be dominated by transient contacts, yet many T cells upregulate the

Immunolgical synapse
The specialized contact area that is formed between a T cell that is interacting with an antigen-presenting cell (APC); it consists of molecules required for adhesion and signalling. This structure is important for establishing T-cell adhesion and polarity, is influenced by the cytoskeleton and transduces highly controlled secretory signals, thereby allowing the directed release of cytokines or lytic granules towards the APC or target cell.

L-selectin
A cell adhesion molecule that is expressed at the surface of most circulating lymphocytes, including naive T cells. It permits lymphocyte homing to the lymph node through high endothelial venules.

Table1 | **Transient versus stable T-cell–DC interactions during antigen recognition in the lymph node**

	Transient contacts	Stable contacts
Duration	3–11 minutes	Several hours
T-cell behaviour during contact	Crawling	Sessile
Calcium signals	Not detected to date	Persistent elevation, irregular spikes of Ca^{2+}
Favoured by	High numbers of regulatory T cells, high precursor frequency of antigen-specific T cells, absence of DC maturation stimulus, PKCθ and the expression of CTLA4	High peptide–MHC density on the surface of DCs, high potency antigenic ligands, high level of DC maturation, ICAM1-mediated adhesion, chemokine bound to the DC surface and the time spent by the T cell in the lymph node

DC, dendritic cell; PKCθ, protein kinase C θ; CTLA4, cytotoxic T-lymphocyte antigen 4; ICAM1, intercellular adhesion molecule 1.

early T-cell activation marker CD69 during this period[35]. Collectively, these experiments indicate that transient contacts with antigen-bearing DCs are one of the distinct modes by which T cells can receive activation signals *in vivo*. Discriminating productive from non-productive short-lived T-cell–DC interactions remains a challenge for future studies.

Stable T-cell–DC contacts in the presence of antigen. Stable, long-lasting interactions between T cells and DCs have been consistently observed *in vivo* using procedures that induce robust T-cell activation. In most instances, these interactions are maintained for the duration of the experiment (TABLE 1), which indicates that the average duration of T-cell–DC contacts largely exceeds that of the imaging period (which is typically 30–60 minutes). The precise duration of these stable contacts is not known and might range from 2–24 hours. *In vitro* experiments support the idea that activation signals can be integrated by the T cells over many hours of TCR stimulation[38–44]. This theory was recently extended based on the results of an *in vivo* experiment that revealed that $CD4^+$ T cells continue to receive signals after 6 hours of prolonged interactions with DCs[45]. Two studies have used calcium-sensitive dyes to detect Ca^{2+} signalling in T cells during interactions with DCs. Stable interactions were associated with an increase in cytosolic Ca^{2+} concentration[37] that could take the form of a persistent occurrence of irregular Ca^{2+} spikes[29]. How this particular pattern of calcium signals affects T-cell activation remains unknown.

Another area of intense research concerns the molecular dynamics at the T-cell–APC junction, also known as the immunological synapse. Antigen recognition by T cells *in vitro* induces the formation of supramolecular activation clusters and dynamic microclusters, which are thought to be important for T-cell activation (reviewed in REF. 46). In addition, a late phase of $CD4^+$ T-cell polarization that occurs after more than 6 hours of stimulation has recently been identified, and was shown to be important for interferon-γ (IFNγ) and interleukin-22 production[47]. Whether all or some of these supramolecular structures are also formed *in vivo* during stable (and possibly transient) T-cell–DC contacts has not yet been explored with two-photon imaging techniques. Investigation of this issue requires the ability to detect the redistribution of relevant fluorescently tagged molecules at the T cell–DC interface in intact lymph nodes. The need for bright fluorescence signals and improved spatial resolution has thus far limited the ability to address this question.

T-cell–DC contacts in the presence of antigen: swarms. Dynamic clusters of T cells 'swarming' around DCs represent another type of T-cell–DC contact dynamics, as observed by Cahalan and colleagues[3,34]. The swarms that were observed consisted of enlarged T cells that displayed looping patterns in their movements and intermittent contacts with DCs. $CD4^+$ T-cell swarms were observed in the late stages of T-cell activation, following the phase characterized by stable interactions, and for a period of a few hours. Such behaviour might provide T cells with additional opportunities to collect signals from DCs.

The sequences of T-cell activation

By synchronizing T-cell activation *in vivo* (BOX 2), von Andrian and colleagues were the first to carefully examine the evolution of T-cell–DC contact dynamics over time[35]. They unexpectedly found that $CD8^+$ T cells and peptide-pulsed DCs primarily established transient interactions during the first 8 hours in the lymph node. It was only after this initial phase of dynamic interactions that stable T-cell–DC contacts were detected. Based on these results, the authors introduced the three

Box 2 | **Synchronizing T-cell activation**

Not all T cells encounter dendritic cells (DCs) at the same time, as new T cells and antigen-bearing DCs can continuously enter lymph nodes. Although this heterogeneity is part of a normal immune response and could have an important role in diversifying T-cell fate[65], it can complicate the interpretation of experiments that are aimed at imaging the kinetics of T-cell–DC interactions. For this reason, strategies have been developed that maximize the synchronization of T-cell activation by DCs. One approach consists of delaying the adoptive transfer of T cells until after antigen-bearing DCs have reached the draining lymph nodes[34]. Because T cells enter lymph nodes as early as 1 hour after transfer, when combined with early imaging, this protocol helps catch the first hours of T-cell activation. In addition, the injection of an antibody against L-selectin a few hours following T-cell transfer further blocks the homing of additional T cells to the lymph node, so that a single wave of T-cell activation can be followed over time[35]. An even more synchronous $CD4^+$ T-cell activation event can be obtained by the transfer of wild-type DCs to MHC class II-deficient recipients[45]. To 'switch on' antigen presentation, the antigenic peptide is injected intravenously, resulting in the loading of the transferred DCs (which are the only MHC class II-expressing cells in the recipient) within minutes. This methodology has been used to track the very first minutes of $CD4^+$ T-cell activation by DCs[45].

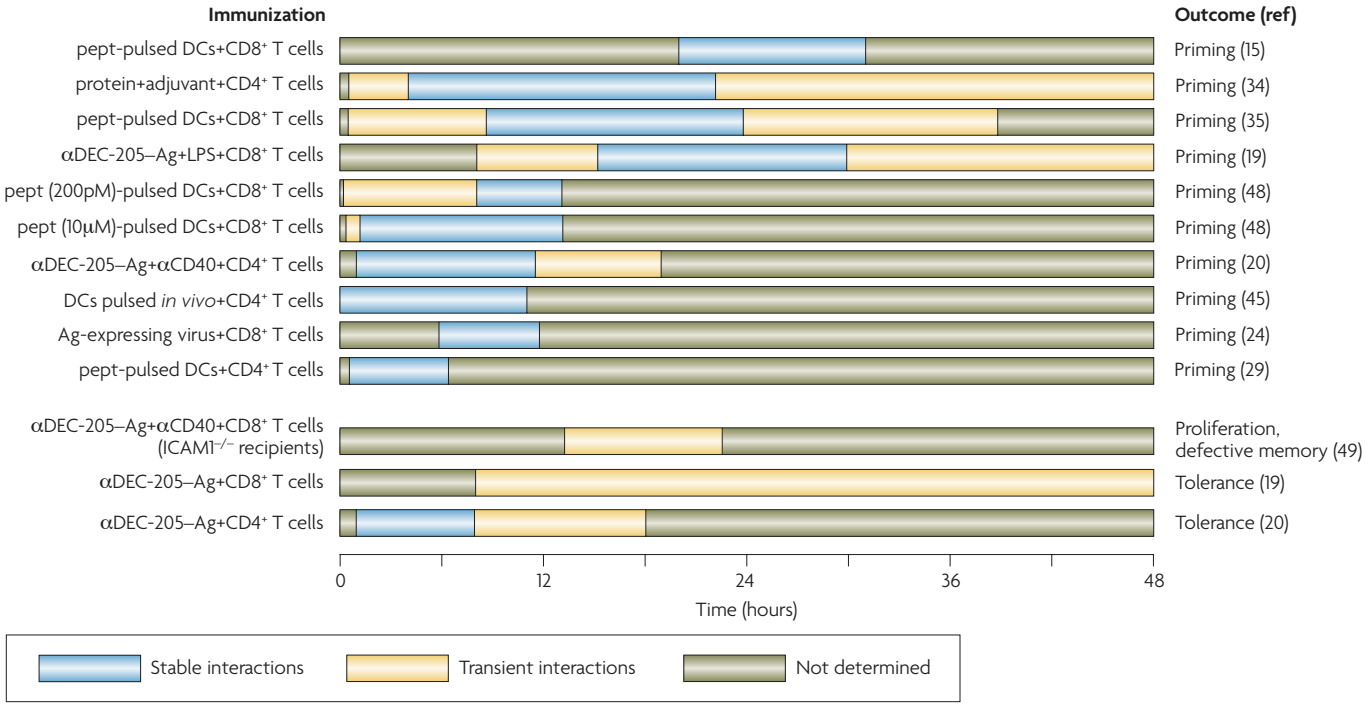

Figure 2 | **Sequences of T-cell–DC interactions during the course of an immune response.** The dominant types of T-cell–dendritic cell (DC) contacts that are observed over the course of T-cell activation in different settings are shown. The various modes of immunization used include injection of peptide-pulsed DCs, injection of antigenic peptide, infection with viruses, or immunization with antigen that is coupled to an anti-DEC-205 antibody (a strategy that targets antigen to DEC-205$^+$ DCs *in vivo*). The outcome of T-cell activation in these various models is indicated on the right hand side. αCD40, CD40-specific antibody; αDEC-205–Ag, antigen coupled to a DEC-205-specific antibody; ICAM1, intercellular adhesion molecule 1; LPS, lipopolysaccharide; pept, peptide.

phases model of T-cell activation, which referred to the ordered occurrence of transient (phase 1), then stable (phase 2), followed again by transient interactions as T cells detached from DCs (phase 3). Two subsequent reports also noted that short-lived interactions were more prominent early in the response to an antigen and were followed a few hours later by the formation of stable T-cell–DC conjugates[19,34]. However, it should be noted that the mode of antigen delivery used in these studies made it possible for the level of antigen presented by the DCs to increase over time. Three other studies failed to detect an initial phase of transient interactions and instead visualized the formation of long-lived T-cell–DC contacts very early in the response[20,29,45]. The dominant patterns of T-cell–DC contacts that were detected over the course of T-cell activation in different models is shown in FIG. 2. Some of the differences in the observed cell dynamics may lie in the diversity of experimental setups used in these different studies. Nevertheless, the emerging consensus is that relatively strong signals might induce rapid or immediate T-cell arrest on DCs. By contrast, T cells might initially transit through a phase of brief contacts with DCs when exposed to weaker signals.

As illustrated in FIG. 3, at least three models can be proposed to explain how T-cell–DC contacts can shift from being transient to being stable over time. One model predicts that changes which occur at the level of the DCs dictate this transition (FIG. 3a). This hypothesis is supported by experiments carried out by Hugues and colleagues[36]. In their experimental settings, CD8$^+$ T cells showed a phase of dynamic interactions with DCs in the first 15 hours following immunization with anti-DEC-205–OVA and CD40-specific antibody (a regimen that induced priming), which was followed by a phase of stable interactions. However, when the DCs were given extra time to mature *in vivo* (by delaying T-cell adoptive transfer by 10 hours), T cells rapidly established stable contacts with DCs, which suggests that DCs but not T cells dictate the timing of T-cell arrest. The increased expression of adhesion and co-stimulatory molecules that accompanies DC maturation is likely to contribute to the gradual ability of maturing DCs to induce T-cell arrest.

By contrast, a second model postulates that T cells 'memorize' signals that are delivered during the initial phase of brief interactions with DCs, and that a certain threshold of signals must be reached before T cells can gain the ability to arrest on DCs (FIG. 3b). Henrickson *et al.* observed that, in the early phase of T-cell activation, antigen-specific CD8$^+$ T cells formed transient interactions with DCs that had been pulsed with a low concentration of an antigenic peptide but established stable contacts with DCs that had been pulsed with a higher amount of the peptide[48]. Interestingly, when a mixture of DC populations that had been pulsed with different peptide concentrations

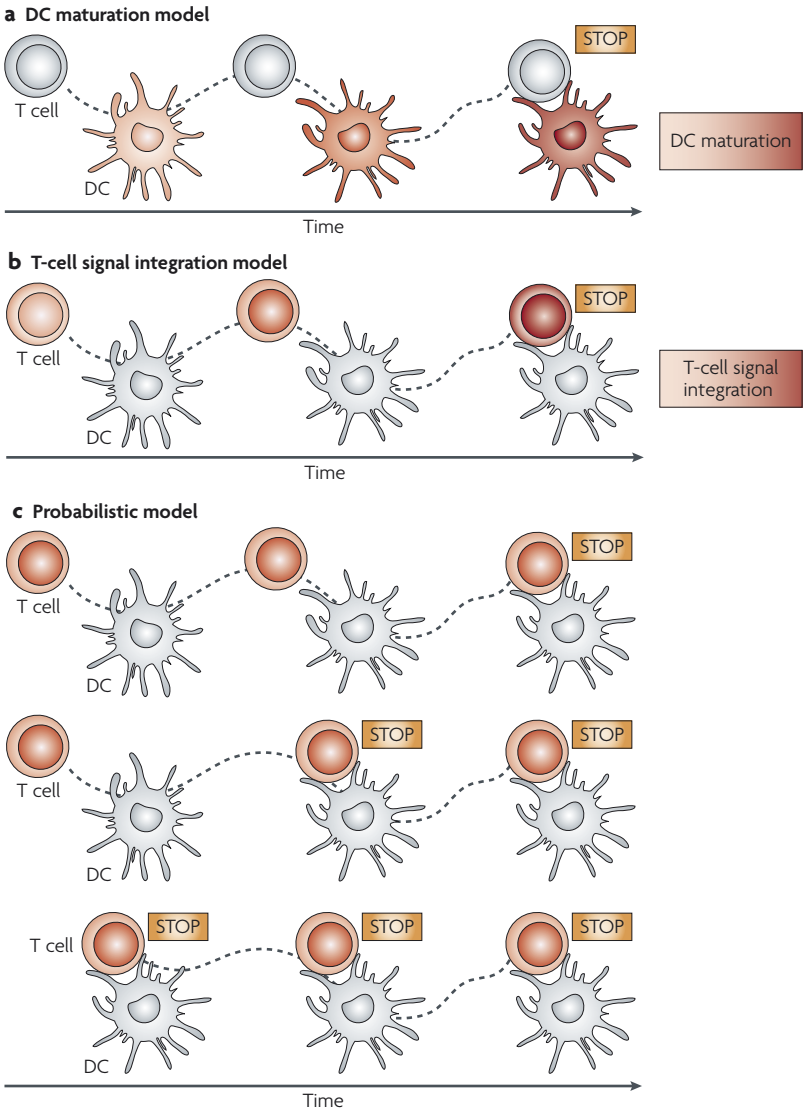

Figure 3 | **Models for the gradual accumulation of stable T-cell–DC interactions over time. a** | In this model, the maturation state of the dendritic cell (DC) dictates T-cell arrest. Over time, DCs gain the ability to establish long-lived interactions with T cells. **b** | T cells sum signals that are received during initial transient contacts with DCs. Once a threshold of received signals is reached, T cells gain the ability to establish stable interactions with DCs. **c** | At each encounter, T-cell arrest on a DC has a certain probability of occurring, which is determined by the overall signal strength. Also, in this model, long-lived T-cell–DC interactions are expected to accumulate over time.

Wiskott–Aldrich syndrome protein
(WASP). WASP is an actin regulator that is involved in the formation of the immunological synapse. Mutations in WASP cause a life-threatening X-linked immunodeficiency that is characterized by thrombocytopaenia with small platelets, eczema, recurrent infections and an increased incidence of autoimmune manifestations and malignancies.

was injected, some $CD8^+$ T cells formed early stable interactions with low-peptide-concentration-pulsed DCs. An attractive interpretation of this experiment is that $CD8^+$ T cells became capable of establishing stable interactions with low-peptide-concentration-pulsed DCs more rapidly, by 'remembering' signals that had been integrated from early encounters with high-peptide-concentration-pulsed DCs.

Finally, a purely probabilistic model could also explain the evolution of contact dynamics over time (FIG. 3c). In this model, T cells have a defined probability to arrest on a DC following an encounter with antigen that is determined by the strength of stimulation and the affinity of the TCR for the antigen. When this probability is low, T cells engage in numerous unsuccessful attempts before arresting on a DC. Because T cells that arrest following antigen recognition initiate a contact that will last for hours, stable contacts are expected to progressively accumulate until they become the dominant type of interactions.

These three models are not mutually exclusive and it is likely that both simple probabilistic considerations and intrinsic changes at the level of T cells and DCs account for changes in contact dynamics over time. However, in all cases, T-cell arrest on DCs occurs preferentially under optimal conditions of antigen recognition, and the various sequences of T-cell–DC contacts might reflect the ability of T cells to choose the best partner to establish a prolonged interaction and to avoid being 'distracted' by DCs that will not provide enough stimulation.

Molecular regulation of T-cell arrest

What is the molecular basis of the formation of stable versus transient T-cell–DC contacts? The amount and the quality of peptide–MHC ligands largely contributes to determining the nature of these contacts. Increasing the number of peptide–MHC complexes on each DC favours the occurrence of stable interactions with antigen-specific T cells[15], and it has been estimated that stable contacts can be formed with DCs that present approximately 100 peptide–MHC complexes[48]. One report classified a set of altered-peptide ligands based on their potency to stimulate $CD4^+$ T cells, and found that high- but not low-potency ligands induced T-cell arrest[37]. Adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1), can also be crucial for T-cell arrest on DCs following antigen recognition. Indeed, the antigen-specific $CD8^+$ T-cell arrest that was observed following the immunization of wild-type mice with anti-DEC-205–OVA and CD40-specific antibody was lost in ICAM1-deficient recipients[49]. Whether variations in ICAM1-expression levels in wild-type mice dictate changes in T-cell–DC contact dynamics remains to be elucidated. Conversely, the expression of cytotoxic T-lymphocyte antigen 4 (CTLA4) by T cells, a co-receptor that profoundly downmodulates T-cell activation, can override T-cell arrest following antigen recognition[50].

Not surprisingly, intracellular signalling molecules also participate in the regulation of T-cell–DC contact stability. Recent results suggested that protein kinase C θ (PKCθ) favours the occurrence of dynamic and short-lived interactions between naive T cells and DCs[51]. This study showed that, during antigen recognition by T cells on lipid bilayers, PKCθ promotes the periodic destabilization of the immunological synapse, which was associated with bursts of T-cell motility. In addition, PKCθ-deficient $CD4^+$ T cells displayed an increased tendency to arrest on APCs *in vivo* following intravenous injection of the cognate antigenic peptide. On the other hand, the Wiskott–Aldrich syndrome protein (WASP), which is an actin regulator, was found to be essential for re-establishing stable synapses *in vitro* after these periodic destabilizations[51].

How are these parameters collectively integrated by T cells? Different studies have provided evidence that Ca^{2+} signals are critical for the induction of T-cell arrest on antigen recognition[37,52]. It is tempting to speculate that the intensity of Ca^{2+} signals, which can be influenced by many factors, reflects the overall strength of stimulation and directs T-cell behaviour during antigen recognition. It is likely that future studies will lead to the identification of new molecules that regulate the stability of T-cell–DC interactions *in vivo*. It will be equally important to determine which of these numerous parameters can be regulated during the immune response and can act as a molecular switch for the formation of stable contacts.

Distinct contacts, distinct outcomes?

Linking the outcome of T-cell activation and contact dynamics. The complex regulation of T-cell–DC contact dynamics suggested that the pattern of interactions that is experienced by an individual T cell may reflect and possibly dictate its function. Studies that compared T-cell–DC dynamics in experimental settings correlated alterations in contact dynamics with different functional outcomes (FIG. 2). Most often, conditions that induced robust T-cell activation were associated with stable interactions and T-cell arrest on DCs. For example, $CD8^+$ T cells arrested on DCs following an immunization regimen that led to T-cell priming (anti-DEC-205–OVA plus lipopolysaccharide (LPS)) but not following an immunization regimen that resulted in T-cell tolerance (anti-DEC-205–OVA alone)[19]. Similarly, a lower level of $CD4^+$ T-cell activation observed in the presence of high numbers of regulatory T cells has been correlated with a reduced stability of $CD4^+$ T-cell–DC contacts[23,53]. Finally, the lack of stable interactions between $CD8^+$ T cells and ICAM1-deficient DCs following immunization with anti-DEC-205–OVA was associated with impaired T-cell memory formation.

However, exceptions to this general trend have also been reported. For example, immunization with anti-DEC-205 that was conjugated to a potent antigenic peptide efficiently triggered T-cell arrest on DCs, yet induced $CD4^+$ T-cell tolerance[20]. Similarly, the formation of stable clusters of $CD4^+$ T cells has been documented following administration of an oral immunization regimen that induced tolerance[54]. In summary, these studies support the idea that stable T-cell–DC interactions are necessary but not sufficient for optimal T-cell priming.

Manipulating T-cell–DC contacts in vivo. An alternative strategy for investigating the extent to which T-cell–DC interaction dynamics shape the outcome of T-cell activation consists of experimentally manipulating T-cell–DC contacts in the lymph node and measuring changes in T-cell activation. A strategy to synchronize the formation and the dissociation of $CD4^+$ T-cell–DC interactions *in vivo* has been exploited recently to identify the T-cell activation profile that is induced by an interaction of a defined duration[45]. At least 6 hours of contact between a $CD4^+$ T cell and an antigen-presenting mature DC were required to initiate clonal expansion, whereas interactions of 24 hours resulted in 5–6 rounds of cell division. These results highlight the importance of prolonged interactions for efficient $CD4^+$ T-cell activation *in vivo*. Moreover, using multiple injections of antigen-bearing DCs, it has also been possible to test the impact of multiple DC encounters on T-cell activation programmes. After their initial activation, $CD4^+$ T cells were shown to be capable of integrating signals during subsequent contacts with DCs, a process that was particularly important for IFNγ production[55].

Heterogeneity of T-cell fates. A common feature of most T-cell responses is the generation of heterogeneous populations of effectors and memory T cells[56,57]. The fact that T-cell fate is closely linked to T-cell–DC contact dynamics raises the possibility that differences in the number and stability of DC encounters that are experienced by different antigen-specific T cells could shape the functional heterogeneity of the T-cell response. This theory has been challenged by an elegant study that analysed the T-cell response mounted by a single antigen-specific T cell adoptively transferred under microscopic control[58]. Although the progeny of each individual T cell was highly diverse, the progenies of different T cells were quite similar to one another, which suggests that diversification of T-cell fate might not originate from differences in initial antigen-recognition events. As strong antigenic stimuli were used in this study, optimal stimulation for all antigen-specific T cells might have been provided. In other contexts, it remains to be established whether differences in the history of T-cell–DC interactions can contribute to the generation of a diverse pool of effector and memory T cells. The recent observation that the initial T-cell division can be asymmetric and that the daughter cells might adopt divergent fates suggests a newly described mechanism for the functional diversification of T cells[59]. Interestingly, asymmetric T-cell division was associated with strong molecular clustering at the immunological synapse, a feature that could be favoured during stable T-cell–DC interactions.

T-cell–DC contacts during infection. Our current knowledge of the dynamics of T-cell activation *in vivo* has been generated with model antigens that were directly targeted to DCs. Although these studies have been instrumental for dissecting spatio-temporal aspects of T-cell–DC interactions, they provide only partial insights into how T cells are primed by DCs during viral, bacterial or parasitic infections. With each pathogen comes a unique combination of parameters that ultimately shapes T-cell–DC communication in the lymph node. Among these are the type, number and localization of antigen-bearing DCs, the cytokine and/or chemokine milieu, the level and kinetics of antigen presentation, and the TCR affinities of the responding T cells.

To take these crucial aspects into consideration, it will be fundamental to unravel the mode and regulation of T-cell–DC interactions during infection with different pathogens. One report analysed $CD8^+$ T-cell priming in mice that had been infected subcutaneously with a vaccinia virus or vesicular stomatitis virus that had both been engineered to express an ovalbumin epitope[24].

T-cell tolerance
The selective inactivation of T cells that are responsive to particular antigens by deleting such T cells, by paralysing them to produce a state of anergy, or by generating regulatory T cells that restrict their activity. The last two effects can occur concomitantly.

Asymmetric T-cell division
This is a process by which two daughter cells can inherit different amounts of immune receptors and signalling components from a parent cell during T-cell division. It has been suggested that this process occurs because of the polarity of the dividing cell that is associated with immunological synapse formation and that it could specify different fates to the progeny of an individual T cell.

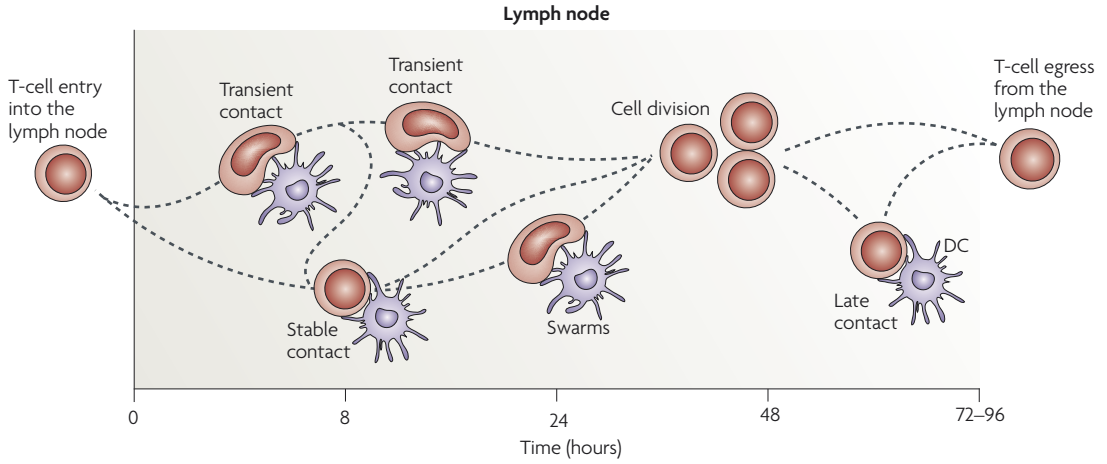

Figure 4 | **T cells can interact with antigen-bearing DCs in lymph nodes in multiple ways.** This figure depicts the various sequences of interactions that T cells can experience during their journey within a lymph node that contains antigen-bearing dendritic cells (DCs). T cells can collect and integrate signals delivered, such as those from the T-cell receptor and co-stimulatory ligands, during each of these encounters. Antigen recognition by T cells can occur through interactions with DCs that are short-lived, long-lived or in swarms. The collection of histories of T-cell–DC contact dynamics probably influences both the quality and the heterogeneity of the ensuing T-cell response.

The lymph-borne virus infected cells that were residing beneath the lymph-node subcapsular sinus which caused a large fraction of antigen-specific $CD8^+$ T cells to redistribute in the peripheral interfollicular regions. $CD8^+$ T-cell–DC interactions were concentrated in these peripheral areas and already appeared to be stable when analysed 6 hours after infection. This is in contrast to other modes of immunization in which T-cell–DC conjugates were found in the vicinity of high endothelial venules (HEVs). This study illustrates that the mode of antigen access to the lymph node can significantly influence the intranodal location of T-cell–DC contacts. As discussed earlier in this Review, T-cell–DC contact stability is both a reflection of the quality of stimulation and a crucial parameter for providing sustained signals. Thus, pathogens might have developed evasion strategies to modulate T-cell–DC contact dynamics and dampen T-cell activation. For example, it has been observed that antigen-pulsed DCs that were treated with the malaria pigment haemozoin were defective at forming long-lasting contacts with antigen-specific $CD4^+$ T cells *in vivo*[60]. Furthermore, OVA-specific $CD4^+$ T-cell arrest following antigen challenge was partially diminished in mice infected with *Plasmodium chabaudi*. Undoubtedly, the conceptual framework that is provided by the study of T-cell–DC interactions with model antigens will be instrumental for further dissecting T-cell–DC contacts during infection.

Concluding remarks

We have learned a great deal by decoding time-lapse movies that are generated by two-photon imaging of T-cell–DC contacts. In lymph nodes, T cells can receive signals during both short-lived contacts and long-lived interactions with antigen-bearing DCs. These contacts are highly regulated events and are influenced by the timing of activation, the signal strength, the inflammatory environment and the presence of other responding T cells (TABLE 1). The integration of these parameters could serve as a quality control mechanism to 'dock' the T cell to an appropriate DC — that is, one which will be able to deliver strong activation signals for the several hours that are required for optimal T-cell activation. Remarkably, T cells appear to sum up TCR signals that are delivered during early and late encounters with DCs, which is a feature that helps them sense the overall amount of antigen available in the lymph node[48]. As a consequence, the quality and the functional heterogeneity of a T-cell response might be shaped by the history of contacts that have been experienced by individual T cells (FIG. 4).

Two-photon movies have not only provided us with a deeper understanding of how T cells collect activation signals from DCs *in vivo*, they have also offered new insights into fundamental mechanisms such as $CD4^+$ T-cell help, intraclonal T-cell competition and the mechanisms of suppression that are used by regulatory T cells. Other important aspects have yet to be explored with this technique, including the role of various DC subsets in orchestrating the immune response and the mode of interactions that is established between DCs and memory T cells. However, the caveat of gathering this unique information has been the use of relatively reductionist experimental approaches. Extending our investigation of these questions to infectious disease models that have close to physiological levels of T-cell precursor frequencies and antigen presentation will be a challenging but important task for the future. In addition, the next revolution for immunoimaging could be reminiscent of the transition from the silent to the sound era of motion pictures. Novel fluorescent probes that track immune-cell functions and signalling pathways will introduce a new dimension to two-photon movies so we can not only look at the cellular choreography of T cells and DCs, but also 'listen' to their molecular 'conversations'. With this in mind, keeping T cells and DCs under the spotlight will continue to unravel fundamental aspects of adaptive immune responses.

High endothelial venules (HEVs). Specialized venules that are found in secondary lymphoid organs, except the spleen, and that are important for lymphocyte homing to these sites. Based on constitutive expression of adhesion molecules and chemokines at the luminal surface, HEVs allow continuous transmigration of lymphocytes.

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Acknowledgements
I thank E. Robey and members of my laboratory for helpful discussions. The work of my laboratory is supported by the Institut Pasteur, Inserm and a Marie Curie Excellence grant.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CCL3 | CCL4 | CCR5 | CCR7 | CTLA4 | ICAM1 | WASP

FURTHER INFORMATION
Philippe Bousso's laboratory web site:
http://www.pasteur.fr/recherche/unites/dri

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