

Translating cell biology *in vitro* to immunity *in vivo*

Marianne Boes* & Hidde L. Ploegh

Department of Pathology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA

(e-mails: Marianne_boes@hms.harvard.edu; ploegh@hms.harvard.edu)

*Present address: Department of Dermatology, Brigham and Women's Hospital, Harvard Institutes of Medicine, Suite 671, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA

The elimination of pathogens and pathogen-infected cells initially rests on the rapid deployment of innate immune defences. Should these defences fail, it is the lymphocytes — T cells and B cells — with their antigen-specific receptors that must rise to the task of providing adaptive immunity. Technological advances are now allowing immunologists to correlate data obtained *in vitro* with *in vivo* functions. A better understanding of T-cell activation *in vivo* could lead to more effective strategies for the treatment and prevention of infectious and autoimmune diseases.

Pathogens are always on the lookout for a host to sustain their life cycle and are an important cause of disease in humans. Because pathogens have a strong preference for particular tissue sites, interacting with or infecting only certain cell types, there is still a paucity of appropriate animal models for the study of human infectious disease. Even where animal models are available, key aspects of host–pathogen interaction cannot usually be observed directly and in real time. With the obvious exception of death or survival (the natural end points), host–pathogen interactions are assessed by measurement of surrogate parameters, such as the number of antigen-specific T cells generated, or the types and levels of cytokines produced. Now that the complete genome sequences of the main human pathogens are available, there is potential to use this information to explore microbial countermeasures that frustrate immune recognition at all levels. New therapeutic principles may then follow.

Since the discovery of the T-cell receptor (TCR) for antigen and the TCR's associated proteins, the exact manner in which this complex array of surface proteins transduces signals has been studied mainly in isolated T cells. The ability to observe the behaviour of T cells and antigen-presenting cells (APCs) in their natural habitat — for example, in lymph nodes or other tissues — is a recent addition to the immunologist's toolkit and has not been fully explored. The importance of an appropriate microenvironment^{1,2} and of signals derived from components of the innate immune system to achieve T-cell activation³ is clear. Here we discuss some of the successive stages of T-cell activation from a cell-biological perspective, highlighting opportunities for translating some of the cell-biological concepts to applications *in vivo*. The design of effective vaccination strategies and the prospects of immune intervention more generally should benefit from a better understanding of how the considerable body of *in vitro* data can apply to an *in vivo* setting.

Subversion of antigen presentation

The evolutionary success of viruses depends on their ability to disarm host defences, or to produce progeny in such overwhelming numbers that innate defences are inadequate and transfer of the virus to another host is a likely outcome. The possible countermeasures are many^{4,5}. Error-prone reverse transcription may allow human immunodeficiency virus

(HIV) to accumulate mutations faster than the adaptive immune system can effectively deal with the antigenically new variants that arise. Viruses with segmented genomes enjoy flexibility in their genetic make-up through a reassortment of the individual segments when the opportunity arises — for example, when a host is infected with two different yet related viruses, such as swine and avian strains of flu. The larger viruses, such as poxviruses and herpesviruses, probably do not have the luxury of simply mutating the gene that encodes a viral antigen. Instead they rely on interference with the cytokine and chemokine networks; they may disarm components of the complement system or interfere with antigen presentation⁶. Many of these arguments apply with equal force to bacterial pathogens⁷. Moreover, Gram-negative bacteria temporarily escape the host's immune system by using a so-called type III secretion system, which mediates translocation of pathogen-derived proteins across the eukaryotic cell membrane. *Yersinia enterocolitica* and *Salmonella typhimurium* deliver a diverse set of virulence factors directly into the cytosol to resist phagocytosis, a requirement for sustained extracellular bacterial replication, as discussed more fully in the review in this issue by Merrell and Falkow (page 250).

The presentation of peptides by major histocompatibility complex (MHC) molecules is essential for an effective and potent immune response, so it is not surprising that examples of interference of class I MHC-restricted antigen presentation are manifold. These include the adjustment of viral antigen synthesis, inhibition of peptide-antigen delivery to the class I MHC products, intracellular detention of newly assembled class I MHC proteins, or even physical destruction of MHC products by re-routing them either to lysosomes or to the cytosol for proteasomal degradation⁸. Too little is known at present about the lifestyle of many of these pathogens to state with precision which strategy is used *in vivo*. A large DNA virus such as human cytomegalovirus may encode over 200 gene products, some 60 of which may be dispensable for virus growth in tissue culture⁹ and are therefore probably determinants of the lifestyle of the virus. It is only recently that bacterial artificial chromosome (BAC)-based technology has become available¹⁰ to rapidly generate collections of deletion and insertion mutants in these large viral genomes. For some of these genes, their function can be determined only in the context of an *in vivo* infectious model, and then only if the

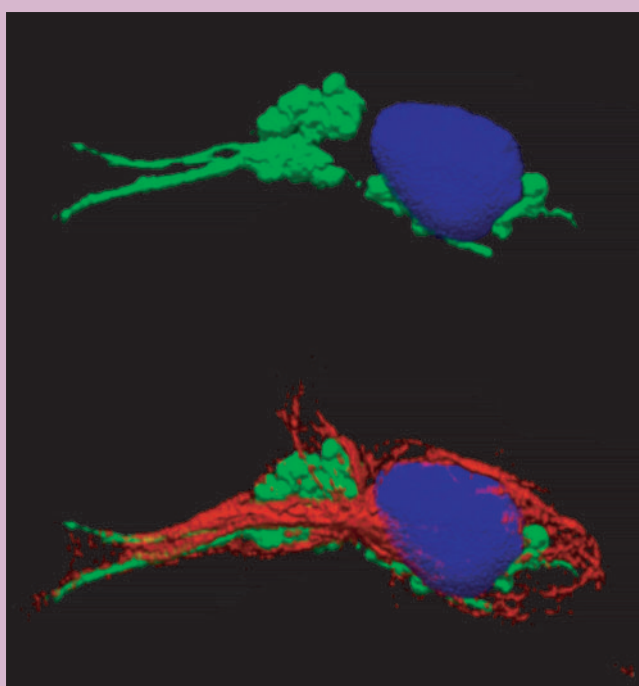


Figure 1 Dendritic cells rearrange their class II major histocompatibility complex (MHC)-positive endosomal compartments in a microtubule-dependent manner upon T-cell ligation. Bone-marrow-derived dendritic cells were grown from mice that express a class II MHC-eGFP (enhanced green fluorescent protein) fusion protein in place of endogenous class II MHC. Class II MHC-containing compartments form tubular structures that are polarized towards antigen-specific T cells. Tubulation was induced by the addition of T hybridoma cells specific for hen egg lysozyme (HEL) peptide⁴⁶⁻⁶¹ to HEL-loaded dendritic cells. In the upper and lower panels, the nucleus is shown in blue (stained with Hoechst 33258); class II MHC-eGFP is in green. Dendritic cells were fixed, permeabilized and stained with antibody to α -tubulin, a key constituent of microtubules. We used a rat anti-mouse Cy3-labelled secondary antibody for detection (red in the lower panel). Reproduced from ref. 27.

appropriate parameters of innate or adaptive/acquired immunity are examined. The resurgent interest in highly pathogenic viruses that could be used for nefarious purposes, such as smallpox, underscores the need for a more thorough analysis *in vivo* of T-cell activation, including the steps immediately preceding it: acquisition of antigen by APCs, the migration of the antigen-laden APCs to the draining lymph node, and their encounter with a naive T cell. *In vivo*, each of these steps presents potential targets for intervention by pathogen-encoded genes.

Transport of antigen

APCs in peripheral immune organs are quiescent sentinels, activated when a pathogen is detected by Toll-like receptors (TLRs) (see review in this issue by Beutler, page 257), scavenger receptors or other surface structures. In particular, dendritic cells continuously survey their surroundings by fluid-phase uptake and endocytosis. Internalization of microbe-derived material induces their differentiation into potent APCs.

Only in lymphoid tissues does priming of naive T cells occur, because naive T cells are usually excluded from non-lymphoid tissues. For example, in the skin, tissue-derived antigens arrive in draining lymph nodes in two ways. During the first 24 hours, transport occurs mainly in free (not in cell-associated) form, in interstitial fluid delivered to the lymph node through afferent lymph vessels. Components of the complement system, in synergy with natural antibodies, help to prepare such antigens for efficient acquisition by the dendritic cell. In the lymph nodes, immature dendritic cells, which form class II MHC-peptide complexes that are used to prime naive T cells,

internalize the first wave of antigen. Thereafter, antigen-laden dermal dendritic cells arrive in the draining lymph node, having acquired their antigenic cargo in the periphery. These skin-derived dendritic cells, having already formed class II MHC-peptide complexes *en route* to their destination, display their MHC-peptide complexes to T cells in the lymph node¹¹. The relevance of these seemingly parallel pathways of antigen presentation must be sought in the heterogeneity of dendritic cells^{2,12}, which may vary in maturation stage, in their proteolytic machinery, and in their expression of costimulatory molecules. Costimulatory molecules may provide stimulatory or inhibitory signals that together control T-cell activation. The difference in the proteolytic capacity of the immigrant versus node-resident dendritic cells would generate class II MHC-peptide complexes that are not necessarily identical, although they derive from the same protein antigen. Different T-cell clones would therefore be activated, leading to more extensive T-cell activation, perhaps including an interconnected network of regulatory T cells.

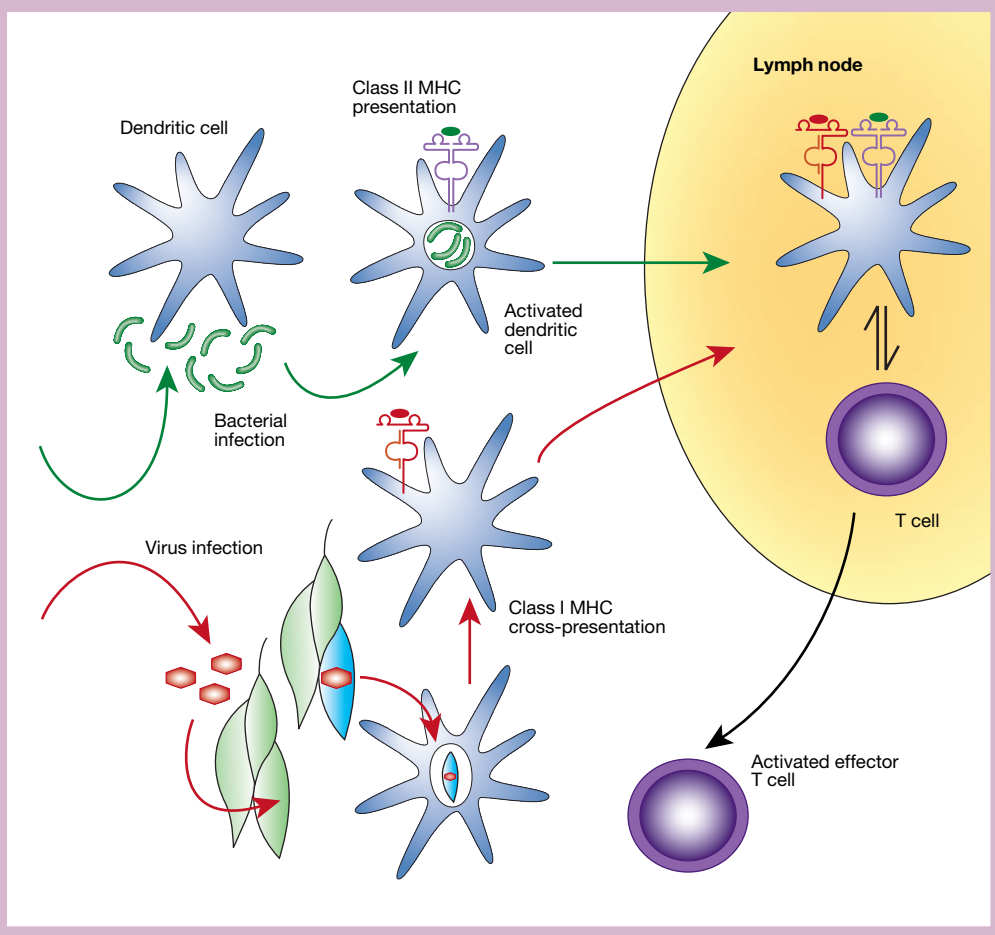
Trafficking of MHC molecules

Proper display of MHC products is a prerequisite for T-cell activation. The efficacy with which a protein antigen is converted to an MHC-peptide complex is not known with certainty, but estimates for a relatively well-studied antigen such as ovalbumin suggest that several thousand protein molecules must be destroyed to generate a single MHC-peptide complex¹³. This has made it technically difficult to track in live cells the conversion of an individual antigen molecule into its corresponding MHC-peptide complex. Perhaps antigen presentation on CD1 molecules, designed to present lipids to T cells¹⁴, could be exploited to examine the fate of synthetic fluorescently labelled glycolipids added exogenously or incorporated into the envelope of bacteria offered to an APC. Although a challenge from the synthetic perspective, the fact that ligands for CD1 are not template-specified, unlike ligands for the classical MHC products, affords possibilities for a chemical-biological approach to the question of how to visualize the ligand for a TCR in a living cell, or even *in vivo*.

Interactions between T cells and APCs can be short-lived, but in many cases contacts between antigen-specific T cells and APCs persist for hours¹⁵. If antigen were in limited supply, such prolonged interaction would imply serial engagement of TCRs by a limited number of MHC-peptide complexes¹⁶. The ability of an APC to carefully regulate this display is probably essential for allowing activation of naive T cells.

Immature dendritic cells are characterized by a paucity of costimulatory molecules, an abundance of class II products in intracellular compartments, and relatively modest quantities of class II MHC molecules displayed at the cell surface. Acquisition of antigen by endocytosis or phagocytosis, under most circumstances in which pathogens are present, will allow the dendritic cells to perceive signals through TLRs. The initial engagement of TLRs initiates a programme of differentiation or maturation¹⁷⁻¹⁹ that includes adjustment of endosomal pH and of active protease content^{20,21}. This increases the ability of the dendritic cell to process the newly acquired antigen and to load the resulting peptides onto class II MHC products. Specialized phagocytes such as macrophages internalize bacteria or apoptotic cells as a normal part of host defence²². Uptake of particulates induces phagosome formation, which by fusion with endosomal/lysosomal vesicles generates antigenic cargo for MHC and CD1 molecules^{23,24}. The fusion of phagosomes with endosomal/lysosomal compartments depends on the presence of TLR ligands²⁵. Phagocytosis of apoptotic cells induces a constitutive mode of phagosome maturation — as characterized by features such as low pH and a strongly reducing and highly proteolytic environment — whereas the complete destruction of bacteria requires the activation of TLRs²⁵. The introduction of phagosome-derived antigenic cargo into loading compartments for class II MHC and CD1 therefore appears to be regulated in part by ligation of TLR ligands.

Figure 2 Presentation pathways of exogenously acquired antigens. Proteins acquired from the surroundings of the antigen-presenting cell can provide a source of peptide for presentation on class II major histocompatibility (MHC) molecules, and in the case of cross-presentation, on class I MHC molecules. Top, bacteria-derived antigens (green) are internalized by dendritic cells and displayed as class II MHC–peptide complexes to antigen-specific CD4-positive T cells in draining lymph nodes. Bottom, dendritic cells can acquire viral antigens (red) by internalization of virally infected tissue (fibroblasts, muscle). Virus-infected tissue cells are proteolysed by the dendritic cells and proteins are processed into class I MHC–peptide complexes to allow activation of naive CD8-positive T cells.



Polarization of class II trafficking in APCs

The generation of green fluorescent protein (GFP)-tagged MHC products using a retroviral delivery system or gene-replacement technology has made it possible to track MHC products in live, professional APCs^{26,27}. This strategy allows examination of the response of APCs to ligands for TLRs, such as lipopolysaccharide (LPS)²⁶, a constituent of the outer membrane of Gram-negative bacteria, and of the APC's response to antigen-specific T cells^{27–29}. Exposure of bone-marrow-derived dendritic cells to LPS results in profound alterations in the morphology of endocytic compartments that are positive for class II MHC. Tubular compartments are formed, possibly by recruitment of internal membrane vesicles from multivesicular bodies³⁰, which are rich in class II MHC molecules. Engagement of an antigen-loaded dendritic cell by a T cell of the appropriate specificity likewise induces the formation of tubular compartments polarized towards the interacting T cell²⁷, a reaction for which previous engagement of a TLR seems to be necessary²⁸ (Fig. 1). What is not clear is whether such responses occur *in vivo*. Both the retroviral and gene-replacement technologies hold considerable promise for the examination *in vivo* of the behaviour of other molecules that control T-cell activation and function. There are obvious limitations to these approaches, because proteins are tagged whose cytoplasmic domains do not easily tolerate such engineered fusions without compromising their ability to transduce signals or interact with partner proteins. Ideally, the functional complementation of a null allele created by gene targeting with the GFP-labelled version of the same gene product would provide the genetic argument necessary to validate this approach. For receptor behaviour in primary cells of the immune system, surprisingly few examples have been subjected to this test *in vivo*. The analysis of interactions between surface proteins and the underlying cytoskeletal elements in primary APCs and T cells would benefit from similarly engineered fusion proteins.

Microdomains

The debate about the nature or even the existence of lipid rafts in live cells is unlikely to subside any time soon^{31–34}. Discovered as membrane specializations that survive extraction in non-ionic detergents and are enriched in glycolipids and in proteins that carry fatty acyl modifications, the size and relevance of these rafts remain unclear. Munro³⁵ raised a number of objections and suggested further experiments to address both the occurrence and the relevance of such membrane specializations. Recent experiments involving fluorescence resonance energy transfer (FRET) measurements suggest a far more isotropic distribution of components that are believed to be raft-specific³⁶, and show that the complexes that comprise these rafts may be far smaller than was originally suggested. Although this is not the place to summarize all the arguments (for reviews, see refs 31, 35), the existence of membrane specializations is easy to accept. Cell biologists are familiar with the distinction between apical and basolateral domains of epithelial cells³⁷ and the pronounced polarization of cells of the nervous system³⁸. But lymphocytes are small and round; they move and change shape, and consequently it is difficult to pin down a cell at any given time without fixation.

Although many investigators use detergent solubility or flotation of extracted materials on sucrose gradients as criteria for localization of their favourite protein(s) to lipid rafts, there are of course other data supporting the existence of plasma membrane microdomains. Lymphocytes and dendritic cells are richly endowed with proteins that belong to the family of membrane proteins called tetraspanins (after their characteristic four transmembrane domains), such as CD63, CD81 and CD9 (ref. 39). Biochemical analyses show that these proteins tend to form complexes with themselves and with other members of the tetraspanin family⁴⁰, and that they recruit other membrane proteins to these tetraspanin webs to form microdomains. Subtle perturbation of membrane structure in dendritic cells abolishes their

ability to stimulate T cells, even when the display of the MHC–peptide ligands themselves is unaffected⁴¹. A combination of intravital imaging methods in conjunction with biophysical techniques such as FRET may help to define the existence and nature of membrane microdomains *in vivo*, both for T cells and for APCs.

Cross-presentation

Many viruses display pronounced tropism, infecting only certain cell types. Even if the virus fails to infect an APC directly, a successful immune response can nonetheless be mounted. This is provided that professional APCs can acquire dying cells whose death may be the consequence of the virus infection (for example, through direct cytopathic effects or by the induction of apoptosis). The ability of APCs to acquire such cell remnants by phagocytosis and then present processed materials derived from these cell remnants by means of class I MHC molecules is called ‘cross-presentation’⁴² (Fig. 2). Mice rendered transgenic for the poliovirus receptor sustain productive infection with poliovirus, a pathogen normally incapable of infecting mice. If these animals are lethally irradiated and reconstituted with bone marrow from donors that do not express the poliovirus receptor, a robust response involving CD8-positive T cells is nonetheless observed. This requires the presence of a functional TAP (transporter associated with antigen presentation) complex in the donor bone marrow cells used for reconstitution, and provides strong evidence that bone-marrow-derived APCs can indeed access the class I pathway of antigen presentation, even when not infected with the virus themselves⁴³. There are at least two possible means by which antigen could be transferred: proteolysed fragments of the antigen or MHC-bound peptides could be liberated from donor cells and then transferred. Escape of such small fragments from a phagosomal compartment might be relatively straightforward and could involve peptide-conducting channels such as TAP, Sec61 or others^{44–47}. Alternatively, whole proteins may be the predominant source of peptides. After transfer to the cytosol, such proteins would undergo ubiquitin-dependent proteasomal degradation. This mode of transfer presumably requires protein-conducting channels with an architecture somewhat different from that of channels used for cotranslational protein import into the endoplasmic reticulum (ER), unless complete unfolding of the protein substrate precedes translocation.

The ability of phagocytic cells to recruit ER membrane to the newly formed phagosome⁴⁸ suggests that the normal constituents of the ER, such as the TAP complex or the Sec61 translocon complex, may be involved in this mode of antigen presentation^{49,50} (Fig. 3). The possibilities for translocation of antigen from the phagosome are by no means limited to these known players, as new escape routes from the ER, and by inference, from phagosomes that have incorporated ER materials, continue to be discovered⁵¹.

If indeed this mode of acquisition is the most important means of delivering antigens for cross-presentation, it follows that those antigens that are abundantly present as proteins will be cross-presented more successfully than the already processed peptides present on donor MHC molecules themselves, or as has been argued, in a complex with members of the heat-shock protein family such as gp96 (ref. 52). New evidence now suggests that this is indeed the case. Elegant experiments show that signal-sequence-derived epitopes or other epitopes predicted to be short-lived are cross-presented poorly, but once grafted onto the body of a carrier protein as a more stable entity, their cross-presentation improves dramatically^{53–55}.

Cross-presentation must be important not only for the recognition of virus-infected cells, but also as an unavoidable consequence of lymphocyte development as well. In the thymus, where the vast majority of newly arising T cells never reach maturity and die by apoptosis, bone-marrow-derived cells in the thymus continuously remove cell remnants. Because such cross-presentation presumably occurs in the absence of an inflammatory stimulus, T cells exposed to these cross-presented materials will be instructed to die, or may never acquire full functional competence and instead become anergic. The

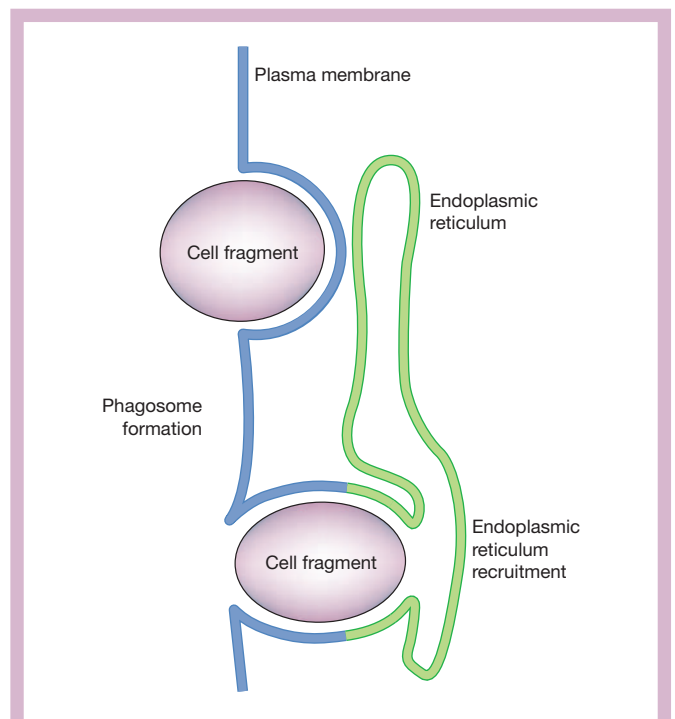


Figure 3 Fusion of phagosome with endoplasmic reticulum membrane. Phagocytic cells (macrophages and dendritic cells) can internalize cell fragments of significant size by recruiting membranes of the endoplasmic reticulum. Internalized exogenous antigens may therefore have immediate access to enzymes and transporters within the endoplasmic reticulum that are involved in class I major histocompatibility complex (MHC) presentation, facilitating cross-presentation.

details of cross-presentation are not yet understood, but experience with the more conventional trafficking pathways of MHC products will provide a useful backdrop.

Activation of T cells by APCs

Activation of T cells occurs when a professional APC displays the appropriate set of ligands to a naive T cell. These ligands must include a proper MHC–peptide complex, as well as the requisite sets of costimulatory molecules. Microbial products, such as cell wall constituents and nucleic acids, trigger members of the TLR family (see review in this issue by Beutler, page 257) and expression of costimulatory molecules. The importance of these surface-displayed costimulatory glycoproteins is apparent from inclusion of the appropriate blocking antibodies, which interfere with the interactions between APCs and T cells. Less clear is the relevance of alterations in cytoskeletal proteins, or in the detailed arrangement of components of the vacuolar system in both the APC and the T cell. Among the early changes seen in an activated T cell is the reorientation of the vacuolar apparatus^{56,57} so that release of cytokines, or the delivery of a lethal hit in the form of perforins and granzymes, will occur in a polarized manner, towards the interacting APC. Rearrangements of the cytoskeletal apparatus and of the microtubule network clearly occur, but how the directionality of these signals is controlled is not well understood. Ion gradients, maintained by controlling influx and efflux, are also under the influence of receptor-mediated events⁵⁸, but compared with the cells of the nervous system, much less is known about the role of ion channels in T-cell activation. Voltage-gated potassium channels do accumulate in the immunological synapse⁵⁹, a structure whose formation requires the ligation of TCRs and a variety of adhesion molecules (see below), but the functional consequences of such recruitment are unclear.

T-cell activation is assessed by monitoring antigen-specific engagement of the TCR, and by examining subsequent receptor-proximal

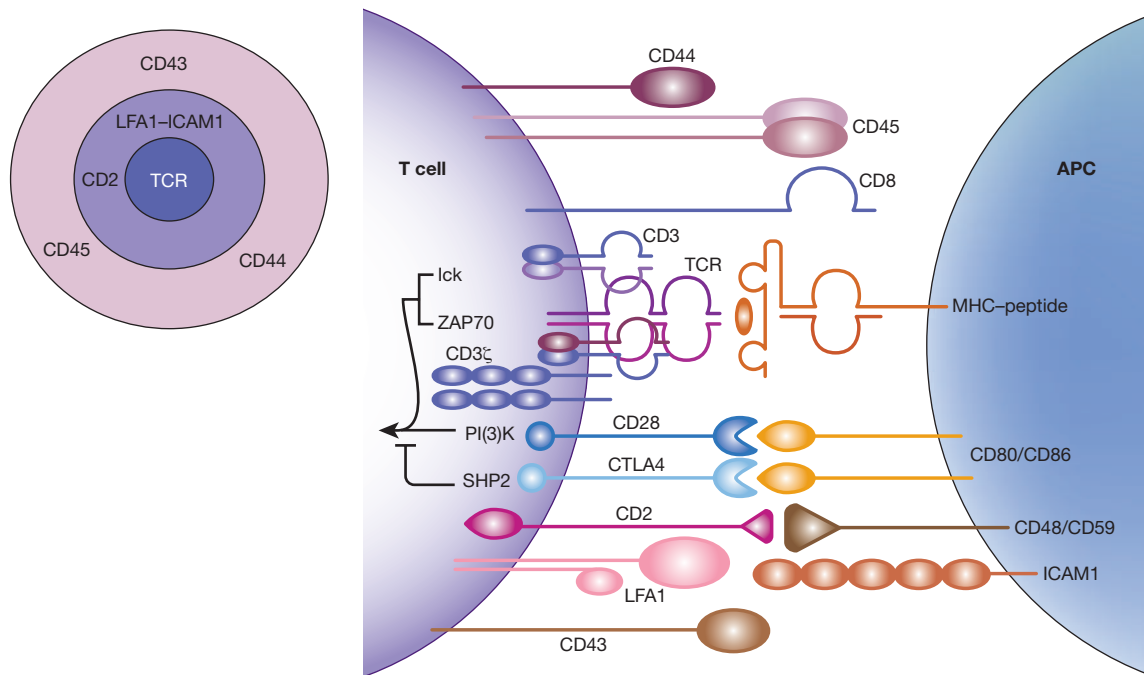


Figure 4 The T-cell synapse. Molecules on a T cell and counterstructures on an antigen-presenting cell (APC) that are known to be involved in T-cell activation are shown. Far left, the face of the synapse with its characteristic distribution of cell-surface molecules, which form a supramolecular activation complex (SMAC). Some of the molecules that are enriched in different regions of the SMAC are shown. The T-cell receptor (TCR) occupies a central region known as the cSMAC; a peripheral ring, the pSMAC, contains adhesion molecules. CTLA4, cytotoxic-T-lymphocyte antigen 4; ICAM1, intercellular adhesion molecule 1; LFA1, lymphocyte-function-associated antigen 1; MHC, major histocompatibility complex; PI(3)K, phosphatidylinositol-3-OH kinase; SHP2, SH2-domain-containing protein tyrosine phosphatase; ZAP70, ζ-chain (TCR)-associated protein kinase, 70 kDa.

events, such as phosphorylation, elicitation of a calcium transient, or alterations in the subcellular localization of key components of the signal transduction machinery⁶⁰. The formation of the immunological synapse begins with integrin-dependent adhesion, closely followed by a redistribution of the TCR, protein kinase C-θ and adhesion molecules^{61,62}. In the APC, the counterstructures for cell-adhesion molecules, the ligand for the TCR and the MHC molecules show similar subcellular redistribution^{42,63,64}. Events that follow include the transcription of diagnostic sets of genes that are turned on, currently measured using genome-wide arrays. For many microbes, the sets of genes expressed under the artificial conditions of the Petri dish differ profoundly from the expression pattern that occurs in the course of a natural infection with that microorganism. T cells maintained *in vitro*, regardless of the exact culture conditions, are also likely to provide at best an approximation of the changes in gene expression that occur when T cells are engaged *in vivo*. Ultimately, T cells acquire functional traits unique to the activated state: the ability to sense the appropriate set of chemotactic cues to reach a site of inflammation, the ability to synthesize and release cytokines that act on T cells themselves and on susceptible target cells within range, or in the case of cytolytic T cells, a licence to kill. T cells are not inert in the absence of a signal delivered by means of the TCR: naive cells perceive chemokine signals and respond to them by leaving the circulation and taking up residence in secondary lymphoid organs⁶⁵. Similarly, not every engagement of the antigen receptor necessarily leads to activation of effector functions: survival signals for T cells include the ligation of TCRs by the MHC products expressed in the host^{66,67}.

Synapses

The concept of the immunological synapse has gained wide currency and its existence is amply supported by observations *in vitro*. Although there is no agreement on their function, supramolecular clusters of diverse surface molecules, as found in the immunological

synapse, must affect the signals transduced by the individual components, even though active signalling molecules rapidly vanish from the synapse⁶⁸ (Fig. 4). The functional distinctions between anergic T cells and their normal counterparts also manifest themselves at the level of synapse formation^{69,70}. In addition, synapses formed by the different types of T cell may depend on the target cell engaged^{68,71,72}, and because of the involvement of non-identical sets of receptors, will also differ between T cells and natural killer cells⁷³⁻⁷⁵, even when similar targets are involved. Whatever their role, whether or not such synapses also form *in vivo* is an important question that has yet to be answered. The architecture of the synapses formed by cells deficient in LFA1 (lymphocyte-function-associated antigen 1) is not known, but because LFA1-deficient mice show no defects in positive and negative selection of developing T lymphocytes⁷⁶, at least the signals transduced in the course of T-cell development can be detected and interpreted in an LFA1-independent manner.

During the initial T-cell priming events, membrane proteins at the cell surface of T cells form organized structures, and corresponding counterstructures occur on the APC. Dendritic cells play an active part in the formation of the synapse. Dendritic cells actively polarize their actin cytoskeleton during interaction with a T cell. The rearrangement of the cytoskeleton in dendritic cells is critical for both clustering and T-cell activation⁷⁷. When B cells are used as APCs, no polarization of their cytoskeleton is seen when cognate interaction with T cells occurs, and pharmacological inhibition of rearrangement of the B-cell cytoskeleton has no effect on B-cell/T-cell conjugate formation or on T-cell activation^{78,79}.

The TCR, MHC-peptide, and costimulation and signal transduction molecules are segregated in the central region of the synapse, whereas molecules involved in adhesion localize to the periphery of the synapse. The TCRs first engage MHC products predominantly by TCR-MHC contacts. Subsequent stabilization of these interactions involves a close apposition of the TCR's CDR3 (complementarity

determining region 3) regions with the peptide cargo of the MHC product. In the course of synapse formation, class II MHC molecules accumulate at the B-cell/T-cell interface in a manner that is dependent on antigen and the expression of costimulatory molecules. The latter may stimulate in T cells the surface display of molecules involved in synapse formation. MHC-peptide complexes that would not by themselves activate the T cell contribute to the formation of a stable synapse and so indirectly support T-cell activation. So far, none of the aspects of synapse formation has been recorded by intravital microscopy, a gap in our knowledge that surely must be filled.

The complexity of interactions between T cells and APCs is compounded by the organization of lymphoid tissue. Cells packed closely together nonetheless manage to migrate at considerable speed, and given the modest numbers of naive lymphocytes specific for any given antigen, it would be remarkable that any successful interactions occurred at all were it not for the precise positional cues conferred by chemokines. Migration of activated T cells in tissues is determined by the chemokine directional code⁸⁰. Although the theoretical framework for chemotaxis and alterations in the direction of migration of motile cells have been well worked out and are supported by experiments *in vitro*^{81,82}, migratory behaviour may be different in a whole animal⁸³. The intraparenchymal migration of live T cells has yet to be visualized *in vivo*. The extent to which homologous and heterologous desensitization of the G-protein-coupled chemokine receptors contribute to the navigation of T cells will also benefit from the emerging techniques for intravital imaging.

Naive T cells generally reside in lymphoid tissues, whereas effector and memory T cells migrate preferentially to tissues that are drained by the secondary lymphoid organs where antigen was first encountered⁸⁰. Dendritic cells from Peyer's patches — a collection of lymphoid tissues located in the mucus-secreting lining of the small intestine — induce the expression in T cells of integrin $\alpha 4\beta 7$ and CCR9, the receptor for gut-associated chemokine TECK/CCL25, to allow effector and memory T cells to migrate to tissue that contains their cognate antigen⁸⁴. Analogous to chemokines, leukotrienes are secreted factors that function as chemoattractants for leukocytes^{85,86}. Leukotriene B4 is a ligand for the high-affinity receptor BLT1 on myeloid leukocytes, and is expressed by effector CD8 T cells, not by naive or central memory T cells. The binding of leukotriene B4 to BLT1 on cytotoxic T lymphocytes is now recognized as a non-chemokine pathway for the migration of CD8 effector cells.

ERM proteins and cytoskeletal elements

The migration of T cells in lymph nodes and other tissues requires not only chemotactic cues, but also the modulation of cell adhesion, and the reorganization of components of the extracellular matrix and cytoskeleton. Members of the widely expressed ezrin-radixin-moesin (ERM) family of cytoskeletal proteins act as general cross-linkers between the cortical actin filament network and the plasma membrane^{87,88}. Their involvement in T-cell activation is well understood and operates mainly through their binding to the cytoplasmic regions of integral membrane proteins such as CD43 in T lymphocytes^{89,90}. Their involvement in APC morphology and function during T-cell ligation is almost unexplored.

In epithelial cells, the ligation of adhesion molecules initially establishes cell polarity⁹¹, which is followed by the organization of the cytoskeleton and sorting of proteins to basolateral and apical compartments^{92,93}. Protein clustering at the cortical membranes occurs, in part, through binding to submembrane scaffolding proteins, which include ERM proteins. Genetic studies in *Drosophila* and *Caenorhabditis elegans* show that ERM orthologues polarize to apical and basolateral compartments and are fixed to the cytoskeleton, where they perform structural and regulatory functions.

Patterns of expression of the three most conserved ERMs are similar in a variety of cells and occur at projections such as microvilli, ruffled membranes, uropods or growth cones. When polarized at these sites, ERM proteins maintain an activated conformation that

links cortical membrane proteins to cytoskeletal actin through conserved amino- and carboxy-terminal ERM-association domains (ERMADs)⁹⁴. The N-ERMAD can bind directly to plasma membrane proteins in the case of ICAM1 (intercellular adhesion molecule 1), ICAM2, ICAM3, CD43 and CD44, or indirectly, through adaptors, which provide docking sites for transmembrane proteins (for example, the cystic fibrosis transmembrane conductance regulator, the β_2 -adrenergic receptor and the sodium-hydrogen exchanger NHE3)⁹⁵.

The cognate interaction of T cells and APCs induces cytoskeletal changes in T cells that appear to adapt the shape of the T-cell surface to that of the APC. Such cytoskeletal changes occur through changes in the phosphorylation status of ERM proteins, which disrupt the anchoring of the cortical actin cytoskeleton from the plasma membrane, and so decrease cellular rigidity to promote more efficient T-cell-APC conjugate formation.

ERM proteins are not the only proteins that anchor surface proteins to cytoskeletal elements. For example, in vertebrate cells an extensive membrane-associated spectrin-ankyrin scaffold conjugates membrane and cytosolic proteins to the actin and microtubule cytoskeleton⁹⁶. Transport mediated by spectrin-ankyrin is important because it facilitates the movement of CD45 (and CD3) to the lymphocyte surface. Proximity of CD45 to the TCR is necessary for the activation of T cells, and defects in CD45 underlie human forms of severe combined immunodeficiency^{96,97}. Activation of T cells by the display of class II MHC-peptide complexes by APCs induces reorganization of the T-cell cytoskeleton, which results in the polarization of the microtubule-organizing center (MTOC). Study of MTOC reorientation in real time has been done in Jurkat cell lines that stably express GFP-tubulin (either proficient or deficient in adaptor molecules involved in calcium mobilization, which is essential for T-cell activation). Calcium-dependent events are required for MTOC polarization towards superantigen-laden B cells, although the players involved in calcium-regulated MTOC polarization are not all known as yet.

Interactions of dendritic cells and T cells in the lymph node

The use of intravital microscopy, sometimes combined with real-time two-photon excitation of fluorophores, has been instrumental in migration studies of T cells *in vivo*^{83,98}. The priming of T-cell responses requires conjugate formation between dendritic cells and T cells; the timely dance between dendritic cells and CD4-positive T cells *in vivo* is now a matter of record¹⁵. During the first eight hours after entry from the circulation, T cells and dendritic cells undergo multiple short encounters, which in the presence of antigen last for about six minutes on average (or four minutes in the absence of antigen). Long-lasting, stable interactions of at least 30 minutes' duration occur during the subsequent 12 hours, during which production of the cytokines interleukin-2 and interferon- γ by T cells is initiated¹⁵. After 24 hours, T cells again have short encounters with dendritic cells, now accompanied by the onset of proliferation and rapid migration. Earlier work on intact explanted lymph nodes indicated that interactions between dendritic cells and T cells are mainly stable in duration, lasting for hours^{99,100}. Maintenance of lymphatic flow, an intact circulatory system, and continued innervation of the tissue examined account for the differences between the intravital and *ex vivo* explant approaches.

Technological advances have put immunologists in the enviable position of being able to correlate an enormous amount of functional data obtained *in vitro* for T cells, B cells and dendritic cells with *in vivo* functions. Being able to manipulate the immune system genetically, and to easily produce radiation chimaeras that involve genetically manipulated haematopoietic precursors, provides opportunities not available for any other organ system. Mice can be created in which lymphocytes, or only some lymphocytes, are the sole cells that harbour a mutation of interest, if necessary introduced as a conditional allele. A thorough understanding of T-cell activation *in vivo* is indispensable

able for the development of better vaccines, for our understanding of the regulatory circuits that break down when autoimmunity causes disease, and for a better understanding of our interactions with the microbial world around us. □

doi:10.1038/nature02762

1. von Andrian, U. H. & Mempel, T. R. Homing and cellular traffic in lymph nodes. *Nature Rev. Immunol.* **3**, 867–878 (2003).
2. Itano, A. A. & Jenkins, M. K. Antigen presentation to naive CD4 T cells in the lymph node. *Nature Immunol.* **4**, 733–739 (2003).
3. Barton, G. M. & Medzhitov, R. Control of adaptive immune responses by Toll-like receptors. *Curr. Opin. Immunol.* **14**, 380–383 (2002).
4. Means, R. E., Lang, S. M., Chung, Y. H. & Jung, J. U. Kaposi's sarcoma associated herpesvirus immune evasion strategies. *Front. Biosci.* **7**, e185–e203 (2002).
5. Vossen, M. T., Westerhout, E. M., Soderberg-Naucler, C. & Wiertz, E. J. Viral immune evasion: a masterpiece of evolution. *Immunogenetics* **54**, 527–542 (2002).
6. Seet, B. T. *et al.* Poxviruses and immune evasion. *Annu. Rev. Immunol.* **21**, 377–423 (2003).
7. Tufariello, J. M., Chan, J. & Flynn, J. L. Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect. Dis.* **3**, 578–590 (2003).
8. Tortorella, D., Gewurz, B. E., Furman, M. H., Schust, D. J. & Ploegh, H. L. Viral subversion of the immune system. *Annu. Rev. Immunol.* **18**, 861–926 (2000).
9. Davison, A. J. *et al.* The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J. Gen. Virol.* **84**, 17–28 (2003).
10. Borst, E. M., Hahn, G., Koszinowski, U. H. & Messerle, M. Cloning of the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome in *Escherichia coli*: a new approach for construction of HCMV mutants. *J. Virol.* **73**, 8320–8329 (1999).
11. 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* **19**, 47–57 (2003).
12. Shortman, K. & Liu, Y. J. Mouse and human dendritic cell subtypes. *Nature Rev. Immunol.* **2**, 151–161 (2002).
13. Princiotta, M. F. *et al.* Quantitating protein synthesis, degradation, and endogenous antigen processing. *Immunity* **18**, 343–354 (2003).
14. Brigl, M. & Brenner, M. B. CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* **22**, 817–890 (2004).
15. Mempel, T. R., Henrickson, S. E. & Von Andrian, U. H. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* **427**, 154–159 (2004).
16. Valitutti, S., Muller, S., Cella, M., Padovan, E. & Lanzavecchia, A. Serial triggering of many T-cell receptors by a few peptide MHC complexes. *Nature* **375**, 148–151 (1995).
17. Inaba, K. *et al.* The formation of immunogenic major histocompatibility complex class II-peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J. Exp. Med.* **191**, 927–936 (2000).
18. Pierre, P. *et al.* Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* **388**, 787–792 (1997).
19. Turley, S. J. *et al.* Transport of peptide MHC class II complexes in developing dendritic cells. *Science* **288**, 522–527 (2000).
20. Lennon-Dumenil, A. M. *et al.* Analysis of protease activity in live antigen-presenting cells shows regulation of the phagosomal proteolytic contents during dendritic cell activation. *J. Exp. Med.* **196**, 529–540 (2002).
21. Trombetta, E. S., Ebersold, M., Garrett, W., Pypaert, M. & Mellman, I. Activation of lysosomal function during dendritic cell maturation. *Science* **299**, 1400–1403 (2003).
22. Aderem, A. How to eat something bigger than your head. *Cell* **110**, 5–8 (2002).
23. Lennon-Dumenil, A. M., Bakker, A. H., Wolf-Bryant, P., Ploegh, H. L. & Lagaudriere-Gesbert, C. A closer look at proteolysis and MHC-class-II-restricted antigen presentation. *Curr. Opin. Immunol.* **14**, 15–21 (2002).
24. Sugita, M., Peters, P. J. & Brenner, M. B. Pathways for lipid antigen presentation by CD1 molecules: nowhere for intracellular pathogens to hide. *Traffic* **1**, 295–300 (2000).
25. Blander, J. M. & Medzhitov, R. Regulation of phagosome maturation by signals from toll-like receptors. *Science* **304**, 1014–1018 (2004).
26. Chow, A., Toomre, D., Garrett, W. & Mellman, I. Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. *Nature* **418**, 988–994 (2002).
27. Boes, M. *et al.* T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* **418**, 983–988 (2002).
28. Boes, M. *et al.* T cells induce extended class II MHC compartments in dendritic cells in a toll-like receptor-dependent manner. *J. Immunol.* **171**, 4081–4088 (2003).
29. Bertho, N. *et al.* Requirements for T cell-polarized tubulation of class II⁺ compartments in dendritic cells. *J. Immunol.* **171**, 5689–5696 (2003).
30. Kleijmeer, M. *et al.* Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J. Cell Biol.* **155**, 53–63 (2001).
31. Cherukuri, A., Dykstra, M. & Pierce, S. K. Floating the raft hypothesis: lipid rafts play a role in immune cell activation. *Immunity* **14**, 657–660 (2001).
32. Horejsi, V. The roles of membrane microdomains (rafts) in T cell activation. *Immunol. Rev.* **191**, 148–164 (2003).
33. Vogt, A. B., Spindeldreher, S. & Kroppshofer, H. Clustering of MHC peptide complexes prior to their engagement in the immunological synapse: lipid raft and tetraspan microdomains. *Immunol. Rev.* **189**, 136–151 (2002).
34. Pierce, S. K. To cluster or not to cluster: FRETting over rafts. *Nature Cell Biol.* **6**, 180–181 (2004).
35. Munro, S. Lipid rafts: elusive or illusive? *Cell* **115**, 377–388 (2003).
36. Glebov, O. O. & Nichols, B. J. Lipid raft proteins have a random distribution during localized activation of the T-cell receptor. *Nature Cell Biol.* **6**, 238–243 (2004).
37. Paladino, S., Sarnataro, D. & Zurzolo, C. Detergent-resistant membrane microdomains and apical sorting of GPI-anchored proteins in polarized epithelial cells. *Int. J. Med. Microbiol.* **291**, 439–445 (2002).
38. Jan, Y. N. & Jan, L. Y. The control of dendrite development. *Neuron* **40**, 229–242 (2003).
39. Tarrant, J. M., Robb, L., van Spruiel, A. B. & Wright, M. D. Tetraspanins: molecular organisers of the leukocyte surface. *Trends Immunol.* **24**, 610–617 (2003).
40. Stipp, C. S., Kolesnikova, T. V. & Hemler, M. E. Functional domains in tetraspanin proteins. *Trends Biochem. Sci.* **28**, 106–112 (2003).
41. Kroppshofer, H. *et al.* Tetraspan microdomains distinct from lipid rafts enrich select peptide MHC class II complexes. *Nature Immunol.* **3**, 61–68 (2002).
42. Heath, W. R. & Carbone, F. R. Cross-presentation, dendritic cells, tolerance and immunity. *Annu. Rev. Immunol.* **19**, 47–64 (2001).
43. Sigal, L. J., Crotty, S., Andino, R. & Rock, K. L. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature* **398**, 77–80 (1999).
44. van Endert, P. M. Peptide selection for presentation by HLA class I: a role for the human transporter associated with antigen processing? *Immunol. Res.* **15**, 265–279 (1996).
45. Wiertz, E. J. *et al.* Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. *Nature* **384**, 432–438 (1996).
46. Johnson, A. E. & van Waas, M. A. The translocan: a dynamic gateway at the ER membrane. *Annu. Rev. Cell Dev. Biol.* **15**, 799–842 (1999).
47. Ackerman, A. L., Kyritsis, C., Tampe, R. & Cresswell, P. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross-presentation of exogenous antigens. *Proc. Natl Acad. Sci. USA* **100**, 12889–12894 (2003).
48. Gagnon, E. *et al.* Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell* **110**, 119–131 (2002).
49. Guermonprez, P. *et al.* ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* **425**, 397–402 (2003).
50. Houde, M. *et al.* Phagosomes are competent organelles for antigen cross-presentation. *Nature* **425**, 402–406 (2003).
51. Lilley, B. N. & Ploegh, H. L. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* **429**, 834–840 (2004).
52. Binder, R. J., Blachere, N. E. & Srivastava, P. K. Heat shock protein-chaperoned peptides but not free peptides introduced into the cytosol are presented efficiently by major histocompatibility complex I molecules. *J. Biol. Chem.* **276**, 17163–17171 (2001).
53. Wolkers, M. C., Brouwenstijn, N., Bakker, A. H., Toebes, M. & Schumacher, T. N. Antigen bias in T cell cross-priming. *Science* **304**, 1314–1317 (2004).
54. Norbury, C. C. *et al.* CD8⁺ T cell cross-priming via transfer of proteasome substrates. *Science* **304**, 1318–1321 (2004).
55. Ploegh, H. L. Immunology: Nothing 'gainst time's scythe can make defense. *Science* **304**, 1262–1263 (2004).
56. Clark, R. H. *et al.* Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. *Nature Immunol.* **4**, 1111–1120 (2003).
57. Kuhne, M. R. *et al.* Linker for activation of T cells, ζ-associated protein-70, and Src homology 2 domain-containing leukocyte protein-76 are required for TCR-induced microtubule-organizing center polarization. *J. Immunol.* **171**, 860–866 (2003).
58. Lewis, R. S. Calcium signaling mechanisms in T lymphocytes. *Annu. Rev. Immunol.* **19**, 497–521 (2001).
59. Panyi, G. *et al.* Kv1.3 potassium channels are localized in the immunological synapse formed between cytotoxic and target cells. *Proc. Natl Acad. Sci. USA* **101**, 1285–1290 (2004).
60. Hogan, P. G., Chen, L., Nardone, J. & Rao, A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev.* **17**, 2205–2232 (2003).
61. Dustin, M. L. The immunological synapse. *Arthritis Res.* **4** (suppl. 3), S119–S125 (2002).
62. Krogsgaard, M., Huppa, J. B., Purbhoo, M. A. & Davis, M. M. Linking molecular and cellular events in T-cell activation and synapse formation. *Semin. Immunol.* **15**, 307–315 (2003).
63. Anderson, H. A., Hiltbold, E. M. & Roche, P. A. Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation. *Nature Immunol.* **1**, 156–162 (2000).
64. Wetzel, S. A., McKeithan, T. W. & Parker, D. C. Live-cell dynamics and the role of costimulatory in immunological synapse formation. *J. Immunol.* **169**, 6092–6101 (2002).
65. Weninger, W. & von Andrian, U. H. Chemokine regulation of naive T cell traffic in health and disease. *Semin. Immunol.* **15**, 257–270 (2003).
66. Ernst, B., Lee, D. S., Chang, J. M., Sprent, J. & Surh, C. D. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity* **11**, 173–181 (1999).
67. Kieper, W. C., Burghardt, J. T. & Surh, C. D. A role for TCR affinity in regulating naive T cell homeostasis. *J. Immunol.* **172**, 40–44 (2004).
68. Lee, K. H. *et al.* The immunological synapse balances T cell receptor signaling and degradation. *Science* **302**, 1218–1222 (2003).
69. Huppa, J. B., Gleimer, M., Sumen, C. & Davis, M. M. Continuous T cell receptor signaling required for synapse maintenance and full effector potential. *Nature Immunol.* **4**, 749–755 (2003).
70. Ehrlich, L. I., Ebert, P. J., Krummel, M. F., Weiss, A. & Davis, M. M. Dynamics of p56lck translocation to the T cell immunological synapse following agonist and antagonist stimulation. *Immunity* **17**, 809–822 (2002).
71. Somersalo, K. *et al.* Cytotoxic T lymphocytes form an antigen-independent ring junction. *J. Clin. Invest.* **113**, 49–57 (2004).
72. Favier, B. *et al.* Uncoupling between immunological synapse formation and functional outcome in human γδ T lymphocytes. *J. Immunol.* **171**, 5027–5033 (2003).
73. Orange, J. S. *et al.* The mature activating natural killer cell immunologic synapse is formed in distinct stages. *Proc. Natl Acad. Sci. USA* **100**, 14151–14156 (2003).
74. Davis, D. M. Assembly of the immunological synapse for T cells and NK cells. *Trends Immunol.* **23**, 356–363 (2002).
75. McCann, F. E. *et al.* The size of the synaptic cleft and distinct distributions of filamentous actin, ezrin, CD43, and CD45 at activating and inhibitory human NK cell immune synapses. *J. Immunol.* **170**, 2862–2870 (2003).
76. Shier, P. *et al.* Impaired immune responses toward alloantigens and tumor cells but normal thymic selection in mice deficient in the β2 integrin leukocyte function-associated antigen-1. *J. Immunol.* **157**, 5375–5386 (1996).
77. Al-Alwan, M. M. *et al.* Cutting edge: dendritic cell actin cytoskeletal polarization during immunological synapse formation is highly antigen-dependent. *J. Immunol.* **171**, 4479–4483 (2003).

78. Kupfer, A. & Singer, S. J. 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.* **170**, 1697–1713 (1989).
79. Wulfig, C., Sjaastad, M. D. & Davis, M. M. Visualizing the dynamics of T cell activation: intracellular adhesion molecule 1 migrates rapidly to the T cell/B cell interface and acts to sustain calcium levels. *Proc. Natl Acad. Sci. USA* **95**, 6302–6307 (1998).
80. Sallusto, F. & Lanzavecchia, A. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol. Rev.* **177**, 134–140 (2000).
81. Foxman, E. F., Kunkel, E. J. & Butcher, E. C. Integrating conflicting chemotactic signals. The role of memory in leukocyte navigation. *J. Cell Biol.* **147**, 577–588 (1999).
82. Wong, M. M. & Fish, E. N. Chemokines: attractive mediators of the immune response. *Semin. Immunol.* **15**, 5–14 (2003).
83. Miller, M. J., Wei, S. H., Cahalan, M. D. & Parker, I. Autonomous T cell trafficking examined *in vivo* with intravital two-photon microscopy. *Proc. Natl Acad. Sci. USA* **100**, 2604–2609 (2003).
84. Mora, J. R. *et al.* Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* **424**, 88–93 (2003).
85. Randolph, G. J. Dendritic cell migration to lymph nodes: cytokines, chemokines, and lipid mediators. *Semin. Immunol.* **13**, 267–274 (2001).
86. Goodarzi, K., Goodarzi, M., Tager, A. M., Luster, A. D. & von Andrian, U. H. Leukotriene B4 and BLT1 control cytotoxic effector T cell recruitment to inflamed tissues. *Nature Immunol.* **4**, 965–973 (2003).
87. Tsukita, S. & Yonemura, S. Cortical actin organization: lessons from ERM (ezrin/radixin/moesin) proteins. *J. Biol. Chem.* **274**, 34507–34510 (1999).
88. Faure, S. *et al.* ERM proteins regulate cytoskeleton relaxation promoting T cell–APC conjugation. *Nature Immunol.* **5**, 272–279 (2004).
89. Delon, J., Kaibuchi, K. & Germain, R. N. Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. *Immunity* **15**, 691–701 (2001).
90. Roumier, A. *et al.* The membrane-microfilament linker ezrin is involved in the formation of the immunological synapse and in T cell activation. *Immunity* **15**, 715–728 (2001).
91. Ojakian, G. K., Ratcliffe, D. R. & Schwimmer, R. Integrin regulation of cell–cell adhesion during epithelial tubule formation. *J. Cell Sci.* **114**, 941–952 (2001).
92. Speck, O., Hughes, S. C., Noren, N. K., Kulikaukas, R. M. & Fehon, R. G. Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity. *Nature* **421**, 83–87 (2003).
93. Bretscher, A., Edwards, K. & Fehon, R. G. ERM proteins and merlin: integrators at the cell cortex. *Nature Rev. Mol. Cell Biol.* **3**, 586–599 (2002).
94. Bretscher, A., Chambers, D., Nguyen, R. & Reczek, D. ERM–Merlin and EBP50 protein families in plasma membrane organization and function. *Annu. Rev. Cell Dev. Biol.* **16**, 113–143 (2000).
95. Short, D. B. *et al.* An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J. Biol. Chem.* **273**, 19797–19801 (1998).
96. Pradhan, D. & Morrow, J. The spectrin–ankyrin skeleton controls CD45 surface display and interleukin-2 production. *Immunity* **17**, 303–315 (2002).
97. Buckley, R. H. Advances in the understanding and treatment of human severe combined immunodeficiency. *Immunol. Res.* **22**, 237–251 (2000).
98. Stein, J. V. *et al.* The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. *J. Exp. Med.* **191**, 61–76 (2000).
99. Bouso, P. & Robey, E. Dynamics of CD8⁺ T cell priming by dendritic cells in intact lymph nodes. *Nature Immunol.* **4**, 579–585 (2003).
100. Stoll, S., Delon, J., Brotz, T. M. & Germain, R. N. Dynamic imaging of T cell–dendritic cell interactions in lymph nodes. *Science* **296**, 1873–1876 (2002).

Acknowledgements We thank J. Cerny, R. Massol and T. Kirchhausen for help with figure 1.

Competing interests statement The authors declare that they have no competing financial interests.