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Receptor clustering and transmembrane signaling in T cells

Jennifer R. Cochran, Dikran Aivazian, Thomas O. Cameron and Lawrence J. Stern

T cells are activated via engagement of their cell-surface receptors with molecules of the major histocompatibility complex (MHC) displayed on another cell surface. This process, which is a key step in the recognition of foreign antigens by the immune system, involves oligomerization of receptor components. Recent characterization of the T-cell response to soluble arrays of MHC–peptide complexes has provided insights into the triggering mechanism for T-cell activation.

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Ligand binding to cell-surface receptors can activate intracellular signal transduction pathways through a variety of mechanisms (Fig. 1). In one common mechanism, typical of seven-transmembrane span receptors that activate G proteins[1], ligand binding induces a structural change in the receptor that can be sensed by effector proteins within the cell. In another mechanism, binding of a multivalent ligand induces receptor colocalization. This mechanism is typical of receptor tyrosine kinases (RTKs), in which receptor clustering facilitates transphosphorylation by the cytoplasmic kinase domains[2]. Other mechanisms driven by mass action can be envisioned (see following text). In a third mechanism, ligand binding induces rearrangement of a receptor oligomer. One example of this mechanism is the bacterial aspartic acid receptor, in which ligand binding induces a helix reorganization that activates receptor-associated cytoplasmic signaling proteins[3]. For soluble ligands, several examples of each of these mechanisms are known. When both the ligand and receptor are cell-surface proteins, the situation can be complicated; for example, by redistribution of membrane components or interaction of other proteins in the juxtaposed membranes. Cases of both of these situations have been observed in the interaction of T-cell antigen receptors (TCRs) with their ligands – major histocompatibility complex (MHC) proteins that are displayed on the surface of antigen-presenting cells. Recent investigation of this cell–cell signaling system, using soluble oligomeric arrays of MHC proteins as mimics of the antigen-presenting cell, have provided insights into the mechanism of T-cell triggering. However, more work is necessary for us to understand the roles of membrane-proximal signaling events in the overall T-cell activation process.

MHC–TCR interaction

T cells play an important role in the initiation and control of immune responses by recognizing antigenic (foreign) peptides bound to MHC proteins on the surface of antigen-presenting cells[4]. MHC molecules bind an extensive variety of peptides from the local cellular environment and display these peptides at the cell surface, providing a diverse peptide library for interaction with T cells. TCRs are generated by clonotypic recombination of genomic constant and

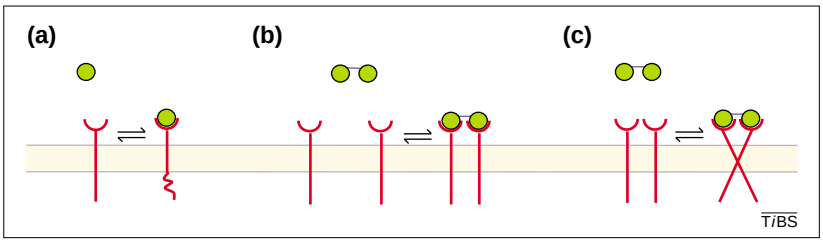

Fig. 1. Mechanisms for transmembrane signaling. (a) Ligand-induced structural change, (b) receptor colocalization, and (c) ligand-induced rearrangement of a receptor oligomer. In principle, each mechanism can be induced by monomeric or oligomeric ligands.

variable gene fragments, combinations of which are selected during T-cell development for functional (MHC-binding) receptors that do not react with self-peptides. Mature T cells circulate throughout the body, examining MHC–peptide complexes on the surface of antigen-presenting cells. Depending on the type of T cell involved and the nature of the interaction, an encounter with a cell displaying antigenic peptides can lead to direct killing of the presenting cell (as for cytotoxic $CD8^+$ T cells), activation of other immune effector cells such as macrophages and B cells (for helper $CD4^+$ T cells), or apoptosis and deletion of autoreactive T cells. This review will focus primarily on signaling in $CD4^+$ T cells.

Initial signaling events in the MHC–TCR system have been difficult to dissect on a molecular level. The intact TCR is a multi-subunit complex comprising six monotopic membrane proteins, $\alpha\beta\gamma\delta\varepsilon_2\zeta_2$ (Fig. 2), with crystal structures available only for the extracellular portion of the antigen-binding $\alpha\beta$ subunits[5]. The MHC–TCR interaction is weak, as determined for the relatively few pairs of MHC–TCR complexes available in soluble recombinant form, with K_d values of ~10^{-4}–10^{-6} M, and lifetimes of ~1–100 sec (Ref. 6). Unlike RTKs, the TCR itself does not possess any intrinsic enzymatic activity. The first detectable biochemical consequence of receptor engagement is phosphorylation of TCR cytoplasmic domains by Src-family tyrosine kinases[7]. Receptor-associated adapter proteins dock onto the phosphorylated cytoplasmic domains, leading to formation of a multicomponent signaling complex containing TCR cytoplasmic domains, adapter proteins, protein and lipid kinases, phospholipases and other proteins[8] (Table 1). Auxiliary receptor–ligand interactions provide co-stimulatory signals, which greatly modulate the character and ultimate outcome of the antigen-specific signal. Neither the initial receptor phosphorylation nor subsequent signaling events have been reconstituted *in vitro*, and T-cell signaling is generally studied in intact T cells.

Soluble MHC oligomers

Soluble crosslinking reagents directed at the T-cell receptor allow receptor-mediated events to be isolated from other cellular processes, and have provided important information about the mechanisms of T-cell activation. Anti-TCR antibodies have long been used to induce characteristic T-cell activation processes[9]. In addition, chimeric T-cell receptors carrying unrelated extracellular domains[10,11], or multiple copies of a cytoplasmic drug-binding domain[12], have been used to show that intermolecular crosslinking of TCR cytoplasmic domains is sufficient to activate some (but not all) signaling pathways, leading to the view that receptor oligomerization is a key step in the activation pathway. However, in studies of T-cell activation, it would be preferable to use the physiological ligands (i.e. the MHC–peptide complexes) rather than antibodies or small chemical ligands. First, serial triggering by individual MHC–peptide complexes, which would allow a single MHC–peptide complex to serially engage and activate multiple TCRs (Ref. 13), has been proposed; this mechanism might not be accessible to a tight-binding compound such as an anti-TCR antibody. Second, the nature of the T-cell response appears to be determined

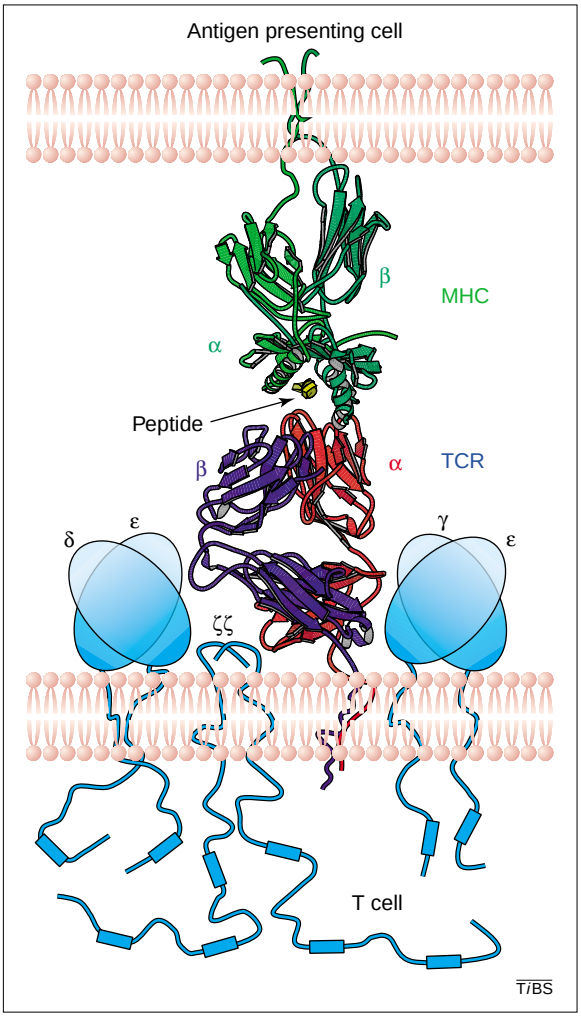

Fig. 2. MHC–TCR interaction. Structures for the extracellular portions of MHC–peptide complexes, TCR$\alpha\beta$ modules and the trimolecular MHC–peptide–TCR complex, are known from X-ray crystallography[5]. TCR$\delta\varepsilon$ and TCR$\gamma\varepsilon$ modules form extracellular disulfide-linked immunoglobulin domains, but no high-resolution structures are available. Stoichiometries other than that shown have been proposed. The cytoplasmic domains of the δ, ε, γ and ζ subunits carry 1–3 copies of the characteristic immunoreceptor Tyr-based activation motif (ITAM, blue boxes) $YxxLx_{6-8}YxxL$. Tyr residues in these sequences are phosphorylated by membrane-associated kinases upon receptor engagement. The relatively large cytoplasmic domains of the $\zeta\zeta$ module might form a membrane-associated helical structure involved in signal transduction[48]. Abbreviations: MHC, major histocompatibility complex; TCR, T-cell antigen receptor.

Table 1. Players in the T-cell activation pathway[a,b]

Protein(s)	Function(s)
MHC proteins	Peptide-binding proteins displayed on the surface of antigen-presenting cells: class I MHC proteins present peptides to $CD8^+$ cytotoxic T cells and class II MHC proteins present peptides to $CD4^+$ helper T cells.
TCR	Multi-subunit, cell-surface complex; the αβ module carries hypervariable extracellular antigen-binding domains that engage MHC–peptide complexes, and the non-variable CD3 δε, γε and ζζ modules carry cytoplasmic Tyr-based signaling motifs (ITAMs).
CD4, CD8	T-cell surface co-receptors for MHC molecules: CD4 binds class II MHC proteins and CD8 binds class I MHC proteins.
CD28, CTLA-4, B7-1, B7-2	Co-stimulatory molecules found on the surface of T cells (CD28, CTLA-4) and antigen-presenting cells (B7-1, B7-2) that deliver activation signals in addition to those from TCR–MHC interaction, and that might play a role in cytoskeletal rearrangements.
LFA-1, CD2, ICAM, CD48	Adhesion molecules found on the surface of T cells (LFA-1, CD2) and antigen presenting cells (ICAM, CD48), which are important in mediating cell–cell contact and in the formation of large-scale supramolecular clusters.
lck, fyn	Membrane-associated Src-family tyrosine kinases responsible for initial phosphorylation of TCR ITAMs upon receptor engagement.
ZAP-70	Cytoplasmic, syk-family tyrosine kinase activated by binding to phosphorylated ITAMs.
CD45	Broad-spectrum protein tyrosine phosphatase that regulates lck activity and might play a role in regulating phosphorylation of TCR subunits.
vav, LAT, SLP-76	Cytoplasmic or membrane-associated adapter molecules, carrying SH2, SH3, phosphoTyr and/or Pro-rich domains, that bind to activated TCR components and engage downstream effector enzymes.

[a]Abbreviations: MHC, major histocompatibility complex; TCR, T-cell antigen receptor.
[b]Roles for many of these molecules in T-cell activation have been reviewed recently[8].

by the affinity between MHC and TCR and/or the lifetime of this complex[6]. For example, single amino acid changes in the MHC-bound peptide substantially influence the quality or nature of T-cell signals induced, and can change an activating peptide complex into an antagonist[14]. Such phenomena are dependent on the specific details of the MHC–TCR interaction, and can only be studied using MHC-based reagents.

Recently, several molecular tools based on oligomerized MHC proteins have been used to investigate the triggering mechanism of T-cell activation (Fig. 3). Oligomers of MHC–peptide complexes were originally developed for use as diagnostic reagents to detect T cells with particular specificities amid the mixed populations present in blood and other clinical samples. Such reagents have become important analytical tools for the cellular immunologist[15], and might be useful as immunomodulatory agents[16]. Soluble, dimeric MHC–peptide complexes have been produced by oligomerization of MHC with antibodies[17] (Fig. 3a) and by fusion of MHC with either antibodies[18] or antibody Fc domains[19,20] (Fig. 3b). Soluble MHC–peptide tetramers have been produced through oligomerization of biotinylated MHC monomers with streptavidin[21] (Fig. 3c), a strategy that can also provide MHC dimers and trimers. Initial studies using these reagents to probe the valency requirements for triggering T-cell activation provided conflicting results, with the apparent minimal number of MHC–peptide complexes in the activating unit determined as one[22], two[17–20] or three[21].

In an alternative approach, a homologous series of MHC–peptide dimers, trimers and tetramers was prepared using synthetic, polypeptide-based crosslinking reagents[23] (Fig. 3d). Incorporation of a fluorescent label into the oligomers allowed simultaneous measurement of MHC binding and T-cell activation by multicolor flow cytometry[24]. Quantitative correlation of binding and activation helped to resolve differences observed in other studies. Higher-valency oligomers activated more potently than did lower valency oligomers, but this was only a consequence of their increased binding avidity; indeed, per MHC molecule bound, T-cell activation was equivalent for an MHC dimer, trimer and tetramer. Monomeric MHC–peptide complexes engaged TCR but did not trigger T-cell activation, confirming that the minimal activating species is a TCR dimer[24].

These results can be used to distinguish between possible models for the mechanism of T-cell triggering. The importance of ligand-induced structural changes within the TCR (Ref. 9) to the activation mechanism (Fig. 1a) appear to be ruled out by crystallographic studies of TCR αβ domains in free and MHC-bound forms, which do not reveal any conformational changes that correlate with activation[25], and by the observation that a substantial fraction of surface TCR can be occupied by MHC monomers without triggering activation[24]. However, detectable changes in T-cell behavior induced by monomeric antibody F_{ab} fragments have been observed, and remain to be explained[9]. The finding that a TCR dimer is necessary and sufficient for signaling is consistent with either TCR colocalization (Fig. 1b) or rearrangement of a pre-existing TCR oligomer (Fig. 1c) being the key triggering event in the activation of T cells by MHC oligomers.

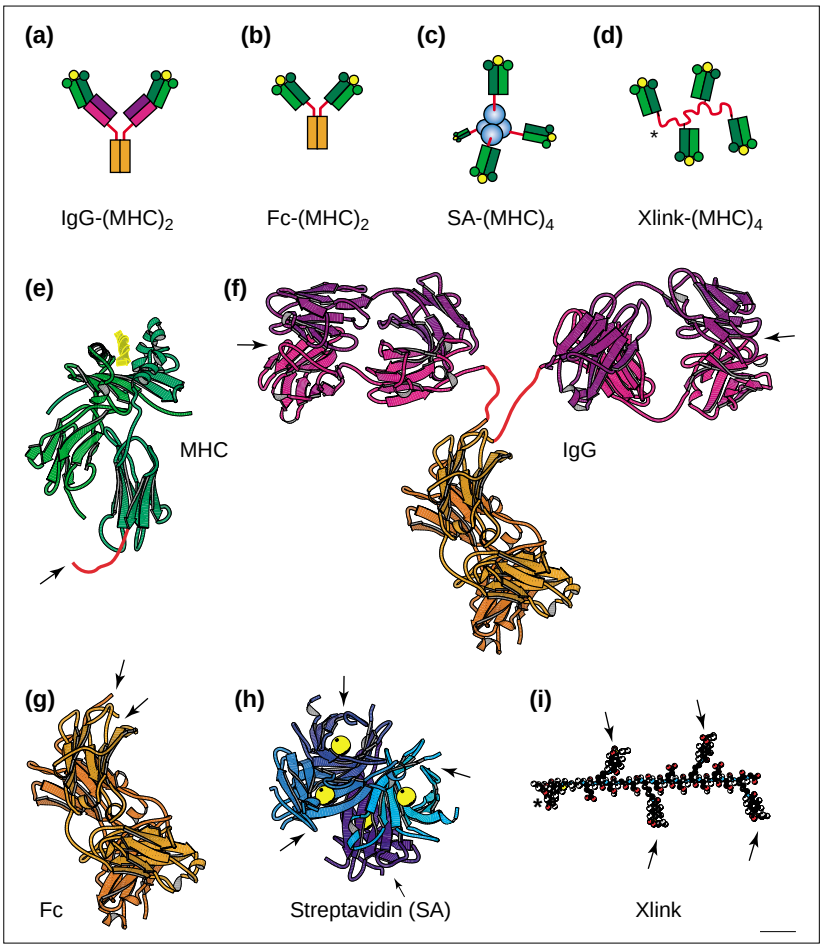

Fig. 3. Soluble oligomeric major histocompatibility complex (MHC)–peptide complexes for studying T-cell activation processes. (a–d) Schematic diagrams of various MHC oligomerization strategies. (e–i) Scale models of oligomer components. Colors are consistent for the various species throughout all panels. (e) Ribbon diagram of the MHC–peptide complex, with αβ subunits in green, and bound antigenic peptide shown in yellow. Flexible β-subunit connecting peptide region (eight residues) is shown in red. MHC oligomers have been made through attachments to the α or β subunit termini, with linkers of up to 12 residues. (f) Antibody (IgG) ribbon diagram with antigen-binding domain (F_{ab}) in purple, constant (Fc) domain in orange and flexible hinge region in red. MHC–peptide dimers have been produced as IgG–MHC complexes (a)[17,18]. (g) Ribbon diagram of antibody Fc domain, used to prepare chimeric Fc–MHC dimers (b)[19,20]. (h) Streptavidin (SA) ribbon diagram (blue), with biotin-binding sites used to link biotinylated MHC–peptide complexes[21] shown as yellow circles. SA-linked MHC dimers, trimers and tetramers (c) have been produced by this method. The exact valency of such complexes can be difficult to determine, particularly for streptavidin–phycoerythrin conjugates, which are large aggregates[26]. (i) Peptide-based synthetic tetrameric cross-linker (xlink) shown as a ball-and-stick model, with reactive maleimide groups used to attach MHC monomers indicated by arrows, and fluorescent probe indicated by asterisk. MHC dimers, trimers and tetramers (d) have been prepared by this strategy[23,24]. All structural diagrams (e–i) are drawn to the same scale. Arrows indicate attachment points.

Several features of the activation process argue for TCR clustering or colocalization (Fig. 1b) rather than rearrangement (Fig. 1c) as being the primary activation mechanism. First, class II MHC oligomers do not bind to most $CD4^+$ T cells under conditions in which cytoskeletal rearrangements are blocked or membrane fluidity is reduced[26], suggesting that multivalent engagement requires the TCR to move or cluster within the plane of the membrane. Second, the inter-receptor orientation does not appear to be crucial for T-cell activation, a point appreciated early on with the realization that many anti-TCR antibodies or chimeric receptor oligomers were sufficient for activation[27], and underscored in a recent study in which many topologically constrained MHC dimers were each able to trigger T-cell activation (Ref. 23 and J.R. Cochran *et al.*, unpublished). By contrast, a ligand-induced TCR rearrangement probably would rely on a particular inter-receptor orientation, as observed recently for the erythropoietin receptor[28]. Third, mathematical analyses of the kinetics of soluble TCR binding to immobilized MHC (Ref. 29), and of the kinetics of MHC-induced TCR internalization measured as a function of antigen density[30], have both suggested that receptor dimerization is a crucial step in the activation process, although the possibility remains that other models could also account for the kinetic results.

Although current evidence favors receptor oligomerization rather than rearrangement of a pre-existing oligomer as the key T-cell triggering process, recent co-precipitation experiments indicate that a substantial fraction of TCR might, in fact, be present as preformed oligomers in the plasma membrane, with two antigen-binding units per complex[31]. Perhaps multivalent binding of such receptor oligomers could lead to higher-order oligomerization as part of a mechanism for expansion of an initial receptor oligomer to large-scale clusters, as observed in cellular experiments[32,33].

Activation by antigen presenting cells

MHC-peptide oligomers and other TCR-clustering reagents have proven useful in investigations of the biochemical mechanism by which TCR communicates binding signals across the membrane. However, on an antigen-presenting cell, MHC proteins carrying a variety of peptides are distributed across the surface, and specific MHC–peptide complexes will probably occur only at a very low frequency. How could interaction with such a cell lead to TCR colocalization and triggering? Although this remains an important question, several mechanisms have been envisaged that could lead to a high density of specific MHC–peptide complexes. Driven by their mutual affinity, both MHC proteins and TCRs would migrate to the interfacial region between an antigen presenting cell and a T cell, thus increasing the density of specific MHC–peptide complexes at the interface[34]. In addition to this passive mechanism, two recent studies suggest that antigen-presenting cells can actively precluster MHC–peptide complexes for interaction with TCRs. Activated B cells have been reported to cluster MHC–peptide complexes into lipid rafts, with disruption of the rafts being deleterious to antigen-presentation activity[35]. (Lipid rafts are low-density, detergent-insoluble membrane microdomains, characterized by high levels of cholesterol, sphingolipids and acylated proteins, which are believed to be sites of intensive signaling activity in several cell types)[36]. In addition, dendritic cells have recently been shown to transport MHC-rich vesicles from endocytic compartments to the cell surface, where they fuse with the plasma membrane, producing MHC-rich membrane patches that are stable for

several hours[37]. The set of MHC molecules in such a patch would probably carry many identical peptides, derived from multiple copies of an antigen present in the endosome. Finally, T cells also appear to have the ability to actively cluster their receptors. Interaction of co-stimulatory molecules on antigen-presenting cells with their T-cell partners (Table 1) has been observed to drive cytoskeletal rearrangements[38,39]. These processes can cluster TCRs at the cell–cell interface (see below), lowering the threshold density for T-cell activation[40]. Any of these active or passive processes might be sufficient to cluster MHC proteins and TCRs at the cellular interface, where specific interactions could then activate signaling processes as observed for the soluble MHC oligomers. However, the possibility remains that T-cell activation by antigen-presenting cells might proceed through a mechanism distinct from that triggered by soluble MHC oligomers[41].

Different activation schemes for CD4+ and CD8+ T cells?

The discussion so far has focused on the activation of CD4+ T cells by class II MHC proteins (Table 1). Although CD4+ (helper) and CD8+ (cytotoxic) T cells generally have been thought to use similar activation strategies, recent studies indicate that their triggering and binding properties could be different. First, careful quantitation of the number of MHC–peptide complexes required for a T-cell response has suggested that a single class I MHC–peptide complex on the surface of an antigen-presenting cell can activate CD8+ T cells[42], but that ~200 class II MHC–peptide complexes are required to activate CD4+ T cells[43]. Second, soluble class I MHC monomers have been reported to be sufficient to induce activation of CD8+ T cells, with activation being dependent on the presence of the co-receptor CD8 (Ref. 22). By contrast, soluble monomeric class II MHC–peptide complexes do not trigger CD4+ T-cell activation, even under conditions in which a large fraction of the total cell-surface TCR are occupied[24], and a crucial role for the co-receptor CD4 in the activation of transmembrane signaling events in CD4+ T cells remains controversial[18,21,33,44]. Third, CD8+ T cells can bind MHC oligomers at 4°C, whereas most CD4+ T cells require elevated temperatures and an active cellular response for binding[26]. Together, these results suggest that a TCR-clustering event is required for activation of CD4+ helper T cells whereas, for CD8+ cytotoxic T cells, CD8–TCR hetero-oligomerization might also play an important role.

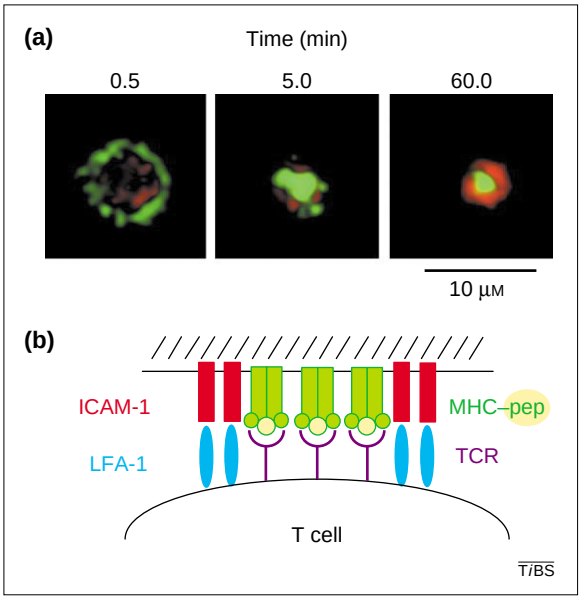

Fig. 4. Large-scale membrane rearrangements during T-cell activation. (a) Fluorescent microscopy of interaction between a T cell and a supported bilayer carrying labeled MHC–peptide complexes (green) and ICAM adhesion molecules (red). The view is normal to the membrane interface. Image adapted with permission from Grakoui *et al.*[33] © 1999 American Association for the Advancement of Science. (b) Schematic diagram of molecular interactions at the immunological synapse. MHC–peptide complexes and TCRs cluster in the center, and are surrounded by adhesion molecules such as ICAM and LFA-1. Video microscopy movies available on the web include: formation of the immunological synapse[33] (http://www.sciencemag.org/feature/data/1040037.shl), cytoskeletal movements and the synapse[49] (http://www.sciencemag.org/feature/data/904937.shl) and differential clustering of CD3 and CD4 (Ref. 44) (http://www.sciencemag.org/feature/data/1050535.shl). Abbreviations: MHC, major histocompatibility complex; TCR, T-cell antigen receptor.

Larger-order clustering

Recently, it has become clear that the interaction of a T cell with an antigen-presenting cell is a much more elaborate process than simple protein–protein contact across juxtaposed membranes, and involves large-scale reorganizations of many membrane proteins into a highly structured and dynamic micrometer-scale region at the cell–cell interface, which has been termed a 'supramolecular activation cluster' or 'immunological synapse' (Fig. 4). Such structures were observed originally by confocal microscopy of conjugates of antigen presenting cells and T cells that had been fixed at various times during the interaction[32], and subsequently by video microscopy of live T cells in contact with a supported lipid bilayer containing MHC–peptide complexes and ICAM proteins (Fig. 4)[33]. This latter approach allows real-time monitoring of the redistribution of MHC proteins, TCRs and other molecules involved in the interaction, using fluorescent proteins either directly labeled and incorporated into the bilayer, or expressed as green fluorescent protein (GFP)-tagged cellular proteins. The immunological synapse forms over the course of ~5–10 min in response to MHC–TCR signaling and co-stimulation, and persists for hours, with TCR and MHC molecules migrating to the center, and adhesion molecules and co-receptors localizing in concentric patterns around the central region[41]. These rearrangements have been imaged in cell–cell as well as cell–monolayer interactions. Mechanisms for maintenance of the immunological synapse in the presence of receptor internalization have been proposed[33]. Formation of the immunological synapse appears to be correlated with induction of the complete cellular activation program, and could function either to integrate MHC–TCR and co-stimulatory signals, or as a specificity checkpoint in which MHC–TCR lifetimes can be evaluated[41].

Biochemical studies have demonstrated that some of the membrane-bound participants in the T-cell activation process, including TCR, lck and CD28, preferentially localize to lipid rafts formed at the interface between the antigen-presenting and T cells[45]. The mechanism that incorporates TCR and other transmembrane proteins into raft microdomains[38] is unknown. These lipid rafts would appear to be related to the supramolecular clusters observed by microscopy but, at present, this connection remains speculative.

Molecular mechanisms

Despite advances in our understanding of the importance of receptor clustering and large-scale membrane rearrangements in the T-cell activation process, the actual molecular mechanism by which clustered TCRs trigger cytoplasmic signal transduction is yet to be understood in detail. Potential triggering mechanisms should be consistent with the observations that TCR cytoplasmic domains alone are sufficient to transduce clustering signals into the cytoplasm, that they have no intrinsic catalytic activity but instead become multiply phosphorylated upon receptor engagement, and that productive engagement does not depend on any particular arrangement or orientation of a TCR oligomer. Several such mechanisms have been proposed. In one potential mechanism, clustering of TCRs serves to increase the concentration of a protein kinase in the vicinity of its substrates: the TCR cytoplasmic domains or associated proteins. The syk-family tyrosine kinase ZAP-70 has been found associated through its phosphotyrosine-binding SH2 domains with a TCR population partially phosphorylated in the resting state in freshly isolated T cells[46], and could play the role of the activating kinase. It was originally thought that lck constitutively associated with CD4 could be the co-localizing kinase; however, observations of CD4-independent activation argue against this interaction as a key activation determinant. In another proposed mechanism, interaction of the relatively small MHC and TCR molecules at the interface of an antigen-presenting cell and a T cell closely apposes their respective cell membranes, sterically excluding proteins with large extracellular domains. In one version of this model, membrane apposition serves to decrease the local concentration of the protein phosphatase CD45 in the vicinity of engaged TCR, tipping the phosphorylation–dephosphorylation balance towards activation[47]. This mechanism has been supported by studies of the effects of variable-sized extracellular domains of CD45 and other cell-surface proteins involved in synapse formation (reviewed in Ref. 41), but is difficult to reconcile with activation by soluble MHC oligomers or other clustering agents. Yet another proposed mechanism is based on the observation of a lipid-induced conformational change in the cytoplasmic domain of the TCR ζ subunit, which regulates accessibility to protein kinases. In this mechanism, clustering would cause a release of the cytoplasmic domain from the membrane, promoting phosphorylation by local kinases[48]. Each of the processes invoked by these proposed mechanisms is likely to occur during T-cell activation, and resolution of the causal relationships among them is sure to be a focus of continuing investigation in this system.

Conclusion

Soluble MHC–peptide oligomers used to investigate the T-cell triggering mechanism have reaffirmed TCR oligomerization as a key event in triggering signal initiation, with the TCR dimer being the proximal activating unit. Further structural and biochemical evidence has suggested that this mechanism occurs through ligand-induced oligomerization of TCR subunits. Large-scale reorganizations of membrane components have been observed at the interfaces of antigen presenting cells and T cells, to form an 'immunological synapse'. How the molecular-scale processes act in the context of the immunological synapse to produce the full cellular activation pathway will be the focus of continuing research. The availability of molecular tools for probing initial MHC–TCR interactions coupled with recent advances in video microscopy should provide insight into the molecular details of this fascinating system.

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Glucose-sensing mechanisms in eukaryotic cells

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Glucose not only serves as a nutrient but also exerts many hormone-like regulatory effects in a wide variety of eukaryotic cell types. Recently, interest in identifying general mechanisms and principles used to sense the presence of glucose has significantly increased and promising advances have been made: in yeast, the first proteins with an apparently specific function in glucose detection have been discovered; in plant cells, there is increasing evidence for a diverse array of glucose-induced signalling mechanisms; and in mammals, glucose-sensing phenomena have turned out to be much more widespread than just in the well-known example of pancreatic beta cells.

Glucose is a ubiquitous nutrient for eukaryotic cells, serving as a source of carbon and energy. In the presence of glucose, a variety of metabolic pathways are affected, resulting in innumerable changes in metabolic intermediates, cofactors and end-products. In addition, different eukaryotic cells use specific mechanisms to sense the presence of glucose (Table 1). The physiological role of these mechanisms depends on the particular cell type.

Yeast cells show a remarkable preference for glucose (and closely related sugars) as a carbon source because these sugars can be converted rapidly to ethanol. The accumulation of ethanol in the medium gives a selective advantage to the highly ethanol-tolerant yeast cells. Hence, yeast have developed mechanisms to rapidly convey the presence of glucose in the medium to the cellular regulatory machinery, ensuring its exclusive and optimal utilization. Genes involved in respiration and metabolism of alternative carbon substrates are repressed, whereas fermentation and growth-related genes are induced[1–4].

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