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Membrane specializations and endosome maturation in dendritic cells and B cells

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Interest in the cell biology of antigen presentation is centered on dendritic cells (DCs) as initiators of the immune response. The ability to examine primary antigen-presenting cells, as opposed to cell lines, has opened a new window for study of antigen processing and peptide acquisition by Class II major histocompatibility complex (MHC) products, especially where intracellular trafficking of peptide–Class-II complexes is concerned. Here, we review the dynamics of Class II MHC-positive intracellular structures in dendritic cells as well as B cells. We focus on the generation of multivesicular bodies, where Class II MHC products acquire antigenic peptide, on the endosomal transport of peptide-loaded Class II MHC to the cell surface and on the importance of Class II MHC localization in membrane microdomains.

Antigen processing and presentation is a catch phrase that describes how foreign materials that can serve as antigens are acquired by antigen-presenting cells (APCs) of the immune system. It is the conversion of mostly pathogen-derived proteins into peptide complexes with major histocompatibility complex (MHC) molecules that is essential for activation of lymphocytes. APCs are defined as cells that constitutively express Class II MHC products. These cell types include dendritic cells, B cells and macrophages. In this review, we focus mostly on dendritic cells (DCs) and B cells. Upon internalization, antigens are converted into a complex of MHC molecules and fragments of these foreign proteins, or into complexes composed of MHC-like products that bind to lipids. The design of the T cell receptor (TCR) for antigen is such that antigen-derived peptides can be recognized by these receptors only when complexed with an MHC molecule. T lymphocytes occur in at least three classes as determined by their ability to recognize peptides presented on Class I MHC, Class II MHC or nonclassical MHC molecules, such as those of the CD1 family that present lipids (Box 1). We will focus here on Class II MHC molecules, which present peptide antigens to the subset of T lymphocytes positive for the CD4 co-receptor. CD4 T cells are involved in establishing an inflammatory environment and also serve as a source of help for B cells [1]. Binding of peptide–MHC products to a specific TCR alone does not activate a T lymphocyte. Molecules on the APC involved in co-stimulation and adhesion need also to ligate counter structures on the same T cell [2,3]. We discuss how the spatial arrangement of these molecules into lipid microdomains on the APC surface is important for T cell activation.

Many microbial products, such as lipopolysaccharide (LPS), flagellins and other cell wall components have inflammatory properties: they are recognized by specific receptors on APCs (i.e. Toll-like receptors, TLRs) and elicit the synthesis and release of mediators (cytokines, leukotrienes) that cause inflammation [4]. Upon receipt of an inflammatory stimulus, provided either by an infectious intruder or in the form of host-derived pro-inflammatory cytokines, DCs downregulate both antigen uptake and retrieval of surface-displayed Class II MHC [5]. Their proteolytic capacity is adjusted as well: foreign material previously acquired is now degraded, not to single amino acids but to peptides, which are the source of peptides loaded onto Class II MHC molecules [6]. After exposure to an inflammatory signal, transport of peptide-loaded Class II MHC and co-stimulatory molecules to the surface of the DC is stimulated [6,7]. These changes are referred to as DC maturation and might coincide with migration of the DC from the peripheral sites of antigen encounter to a draining lymph node. It is here that the DC interacts with a naïve antigen-specific T cell [8].

During DC maturation, the display of peptide–Class-II MHC complexes on the cell surface increases dramatically [9]. A large variety of peptides are presented, whose distribution at the cell surface is not necessarily random or homogeneous. For the activation of naïve T cells, multiple interactions between peptide–MHC and TCR are required, where many TCRs interact with a more limited number of peptide–MHC complexes [10], so that each peptide–MHC complex can trigger more than a single TCR on that T cell. Approximately 200–300 peptide–Class-II MHC complexes, which account for a mere 0.002–0.006% of the total surface-exposed Class II MHC, are estimated to be required to fully activate a naïve CD4 T cell so that it acquires effector functions in immune defense [11,12]. In lipid bilayers that contain peptide–Class-II MHC complexes that can diffuse freely, a density of 0.2 peptide–MHC complexes per μm^2, equivalent to 100–200 peptide–MHC complexes per APC, was sufficient to

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Box 1. Class I MHC, Class II MHC and CD1

Class I MHC, Class II MHC molecules and nonclassical MHC-like CD1 molecules (Figure I) sample different pools of antigens: proteins (Class I, Class II MHC) and glycolipid breakdown products (CD1). Their cell-surface display allows recognition by T cell receptors.

Class I molecules consist of two noncovalently linked polypeptide chains: an MHC-encoded heavy chain of 44 to 47 kDa and a non-MHC-encoded 12 kDa subunit, β2-microglobulin. Class I molecules are constitutively expressed on most nucleated cells. This pattern of MHC expression is linked to the function of Class-I-restricted $CD8^+$ killer T cells, which recognize Class I MHC–peptide complexes presented on the surface of, for example, virus-infected cells. Most nucleated cells can sustain replication of viruses. Class I MHC-associated peptides are produced by the proteolytic degradation of cytosolic proteins. In tumor cells, mutated 'self' genes or oncogenes can yield peptide antigens that are recognized by Class-I-restricted cytotoxic T lymphocytes (CTLs).

Class II molecules comprise two non covalently associated polypeptides chains, an α chain (32 to 34 kDa) and a β chain (29 to 32 kDa), both encoded by polymorphic MHC genes. The invariant chain (Ii), a type II membrane protein, associates with newly synthesized Class II molecules. Ii promotes folding and assembly of Class II molecules and directs them to the endosomal/lysosomal pathway. Most Class-II-associated peptides are derived from endocytosed protein antigens. Within Class II MHC-containing compartments, Ii is degraded by lysosomal proteases, the same enzymes that also generate antigenic peptides from internalized antigens. Class II molecules are stabilized by bound peptides, and the complexes are delivered to the surface of the APC, where they are displayed for recognition by $CD4^+$ T cells.

CD1 molecules are β2-microglobulin-associated Class I MHC-complex-like proteins encoded by non-MHC genes. There are considerable differences between species in the number of CD1 genes. The CD1 heavy chain contains sorting information in its cytoplasmic tail for delivery to endocytic compartments where loading with lipid antigens occurs. The antigen-binding domain of CD1 presents lipid antigens to an unusual specific T lymphocyte subset, so-called CD4 +, NK1.1 + lymphocytes that produce IL-4 in response of T cell receptor (TCR) activation. Ligands presented by CD1 might be supplied through an exogenous pathway (phagocytosis).

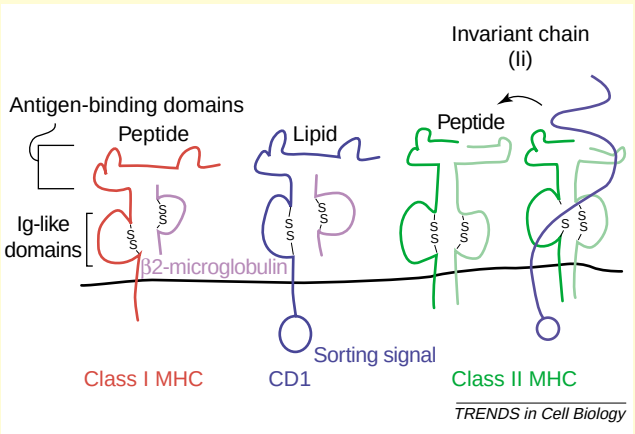

Figure I. Schematic representation of Class I MHC, Class II MHC and CD1 molecules. Class I MHC and CD1 complexes contain a glycoprotein heavy chain and the light chain, β2-microglobulin. Class II MHC complexes consist of an α and β chain. The CD1 heavy chain contains an endosomal sorting motif, whereas Class II αβ complexes are sorted to endosomal compartments through association with Ii. Sorting signals in CD1 and Ii are indicated by a circle.

initiate T cell activation and proliferation [13,14]. Still, the earliest signs of TCR engagement are seen at far lower numbers of successful MHC–peptide recognition events, as exemplified by the encounter of cytotoxic T lymphocytes with a target cell [15]. Most of this information comes from experiments performed *in vitro*. How would a T cell within the confines of a lymph node manage to accumulate all the information necessary for its activation, under conditions where MHC–peptide complexes are in short supply? As we discuss below, the formation of membrane microdomains might be an important contributing factor.

Class II MHC molecules acquire peptide cargo in endosomal compartments (Box 2), which include multivesicular bodies (MVBs). The lipid composition of MVB membranes is characterized by a high cholesterol content [16]. Organization of discrete lipid microdomains might help preserve an adequate local density of Class II MHC molecules loaded with peptides derived from the same antigen as well as that of accessory molecules involved in adhesion and co-stimulation [17,18]. The formation of such clusters might be initiated in Class II MHC-positive endosomal compartments, and the mode of delivery of Class II MHC molecules to the cell surface might ensure their persistence after fusion with the cell membrane. How peptide-loaded Class II MHC complexes are transported to the cell surface is not completely understood. In immature DCs and B cells, Class II MHC molecules occur primarily in vesicular compartments. In activated DCs, endosomal compartments often transform into tubular structures. One model is that, in DCs, the contents of an antigen-loaded endosome might be sampled by directing tubules from the endosome to the cell surface [19–22]. These tubules might travel along pre-existing microtubule tracks, deliver peptide-loaded Class II molecules to the cell surface and so form a conduit between the Class II loading compartment and the cell surface [21].

An initial perusal of the peptide–Class-II MHC offerings of a DC would allow the T cell to interact with these microdomains, making use of its TCR and engage other molecules found on the target DC in these microdomains. This contact then mobilizes further Class-II–peptide complexes from the internal stores in the DC that originally provided them. Making use of the microtubule tracks already laid down, tubules emanating from the intracellular Class-II-positive compartment then deliver a steady supply of Class II molecules to the DC–T-cell interface [21] (see model, Figure 1). The combined presence of peptide-loaded Class II MHC molecules with intercellular adhesion molecules ICAM-1 and ICAM-3 and co-stimulatory molecules such as CD80 and CD86 in the same microdomains [7] appears essential to activate naïve antigen-specific CD4 T cells.

Biosynthesis of class II MHC products

APCs continuously sample their surroundings by endocytosis. After internalization, antigens enter acidic endosomal compartments, where protein antigens are processed to yield peptide substrates for Class II MHC products [23]. Contact between antigenic peptides and Class II MHC molecules is ensured by the intersections of the endocytic and biosynthetic-secretory pathways [24]. Class II MHC

Box 2. Antigen processing compartments for Class I and Class II MHC

The major mechanism for the generation of peptides from cytosolic protein antigens is proteolysis by the proteasome (Figure I). Peptides generated by the proteasome are translocated into the endoplasmic reticulum (ER) by a specialized transporter, the Transporter associated with Antigen Processing (TAP). Within the ER, newly formed Class I dimers interact with the TAP complex through an MHC-encoded chaperone, tapasin. Upon binding of peptide, the peptide–Class-I complex is released from the tapasin and is displayed at the cell surface.

Class II MHC molecules present peptides derived from endocytosed antigens degraded by endosomal/lysosomal proteases. The characterization of Class II MHC-positive compartments has received considerable attention but suffers from, at times, confusing nomenclature. There is an emerging consensus that compartments referred to as CIIV are located early in the endocytic pathway, whereas structures that have been called MIIC are positioned later in the endocytic pathway [86,87]. CIIV include transport vesicles that deliver Class II MHC molecules from late endocytic compartments to the cell surface.

An important distinction between the Class I MHC and Class II MHC peptide loading pathways is topological: cytoplasmic antigens are presented through the former, endocytosed antigens primarily through the latter. Dendritic cells are capable also of cross-presentation, often linked to phagocytosis. Cross-presentation results in the delivery of molecules acquired from the extracellular environment to the Class I presentation pathway [88]. In this way, remnants of virus-infected cells can be acquired by an APC, which might then activate $CD8^+$, Class I MHC-restricted killer cells.

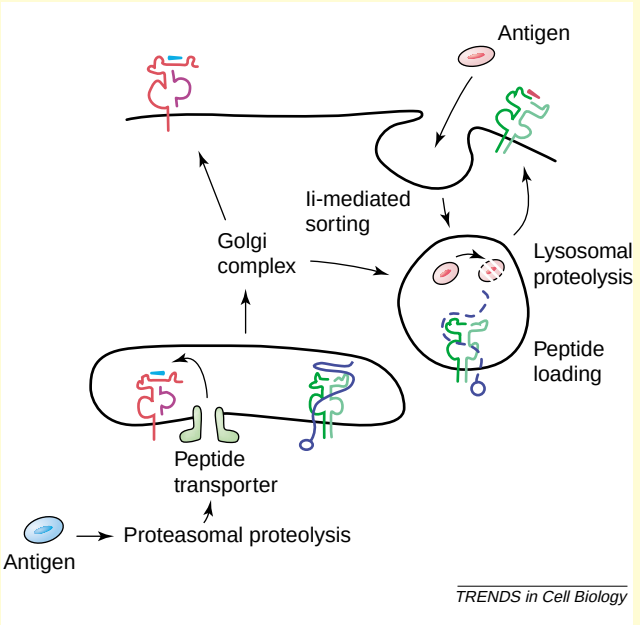

Figure I. Scheme of antigen loading of Class I and Class II MHC molecules. Both Class I MHC and Class II MHC molecules are assembled in the endoplasmic reticulum (ER), whereas their respective peptide cargo is acquired in different subcellular compartments. Peptide substrate for Class I MHC is generated by proteasome-mediated protein degradation. Peptides are introduced in the ER by TAP-mediated transport for loading onto Class I MHC molecules. Class II MHC complexes acquire antigen cargo internalized by endocytosis in endocytic compartments.

molecules are αβ dimers assembled in the endoplasmic reticulum (ER), where the Class II MHC peptide-binding groove is occupied with a segment of the chaperone invariant chain (Ii) [25]. Homotrimeric Ii functions as a scaffold for proper folding of Class II αβ dimers, to yield nonameric complexes of homotrimeric Ii on which three αβ dimers are assembled. The cytoplasmic tail of Ii mediates sorting through leucine-based sorting signals, but possibly in a protein-kinase-C-dependent manner as well, of $(\alpha\beta\text{-Ii})_3$ Class II MHC complexes from the *trans*-Golgi network into the endocytic pathway [26,27].

The localization of Class II MHC molecules and their accessories is non-random; while the internal vesicles of a MVB are rich in Class II MHC molecules, the distribution of the non-classical Class II dimer, the exchange factor H-2M, appears to be preferentially on the delimiting membrane [19]. During transport of Class II MHC molecules, Ii is progressively proteolysed, until only a small fragment called the Class-II-associated invariant chain-derived peptide (CLIP) remains. For most Class II MHC alleles, interaction with H-2M is required to catalyze the release of the Ii-remnant CLIP from Class II MHC molecules and to expose the ligand-binding groove for peptide binding [28]. Those peptides bound by Class II MHC that can no longer be dislodged by H-2M are the peptides displayed at the surface as Class-II–peptide complexes.

Inflammatory stimuli regulate degradation of protein antigen and formation of Class II complexes loaded with antigen-derived peptide [6,29,30] and in DCs also induce the transformation of endosomal compartments (Figure 1). B cells are a second category of APCs. They too rearrange their endocytic compartments when triggered by ligation of the B cell antigen receptor (BCR). Not only is there recruitment of Class II molecules to lipid rafts [31,32] (in fact, it has been argued that they reside there constitutively [33]), but there might also be increased formation of MVBs [34]. Whether the newly formed MVBs generate yet other specialized structures to facilitate antigen presentation by B cells is not known.

Formation of multivesicular bodies

Activation of DCs stimulates the formation of peptide–Class-II complexes [6] and transformation of MVBs into tubular endosomes [19], whereas *de novo* formation of MVBs in DCs has not been observed. Activation of B cells, however, does induce MVB formation. The level of antigen capture and uptake by a B lymphocyte is dictated mostly by the specificity of its clonotypic BCR [35]. Upon BCR ligation, *de novo* formation of MVBs is induced in a manner dependent on phosphoinositide 3-kinase, which then enables B cells to become more efficient APCs [34,36]. Engagement of the BCR results in differential activation signals and B cell responses, depending on the affinity with which the antigen binds, and of course on the number of BCRs that have been engaged [32,37]. The binding of antigens that bind to multiple BCRs induces the formation of BCR clusters or caps [38] and the movement of BCRs into lipid rafts [39,40]. Activation of B cells by ligation of multiple BCRs might have a stimulatory effect on the formation of peptide–Class-II MHC complexes as well.

Sorting of membrane proteins into MVBs has been studied in detail in the regulation of epidermal growth factor (EGF) responses [41]. The sorting of Class II MHC molecules follows a similar pattern, as both the EGF receptor and Class II MHC molecules are stored

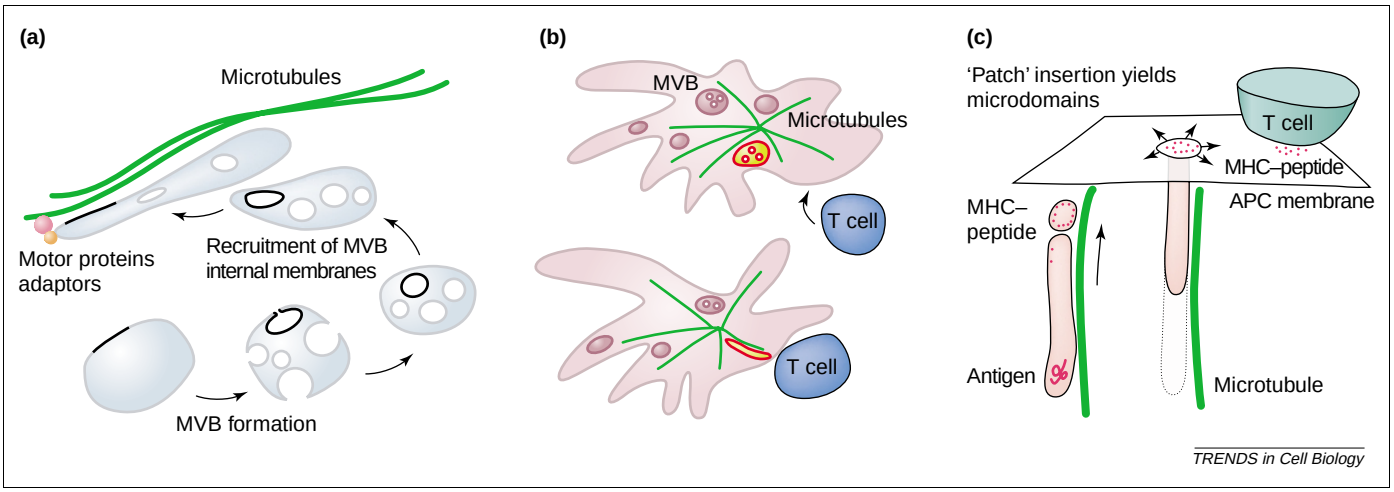

Figure 1. Schematic representation of multivesicular body (MVB) formation and recruitment of internal MVB membranes. Multivesicular bodies are formed through invagination of the delimiting membrane. Internal membranes might be recruited to the delimiting membrane in a signal-dependent manner. Tethering of these endosomal structures to microtubules might allow microtubule-associated adaptor and motor proteins to elongate these structures to generate tubular endosomal compartments of considerable length **(a)**. The signals that evoke conversion of MVBs into tubular compartments might be initiated by contact of the antigen-presenting cell (APC) with an antigen-specific T cell **(b)**. Upon fusion of a tubular endosome or a vesicle derived from it with the plasma membrane, insertion of specialized regions (microdomains) at the plasma membrane might occur. These microdomains might improve the ability of the APC to stimulate the antigen-specific T cell **(c)**.

temporarily in MVBs before transit to a different compartment. For Class II MHC molecules, maturation stimuli induce transport to the cell surface [42], whereas the EGF receptor is eventually degraded in lysosomes. The generation of peptide substrates for Class I MHC is rather inefficient (~4000 proteins destroyed to yield a single peptide–MHC complex) [43]. This might be the case for generation of Class II MHC–peptide complexes as well and leads to the prediction that most internalized proteins experience the same fate as the EGF receptor: complete destruction in lysosomal compartments. Fusion of the MVB delimiting membrane with lysosomal structures allows exposure of EGF–EGF receptor complexes to hydrolytic enzymes resulting in their complete degradation as a means to downregulate the cellular response to EGF [44,45]. The complexity of MVB sorting is illustrated by the number of gene products that are involved: more than 50 gene products are likely involved in vacuolar protein sorting in yeast [46] and 15 Class E Vacuolar protein sorting (Vps) proteins are necessary for sorting proteins into MVBs [47,48]. New developments in MVB sorting are summarized in recent reviews [49,50]. Our understanding of MVB sorting has yet to be applied in full to the analysis of Class II MHC-restricted antigen presentation, but the fate of the EGF–EGF receptor complexes is likely to serve as a useful example.

A point mutation in the EGF receptor cytoplasmic domain abolishes its transfer to internal vesicles of MVBs [51] and degradation of the EGF receptor in internal vesicles is mediated by ubiquitin ligation to the cytoplasmic domain of the EGF receptor [52,53]. The yeast Class E Vps23 protein and its mammalian homolog, Tsg101, have ubiquitin-conjugating (UBC)-like domains that bind to ubiquitin [54–56]. Both Vps23 and Tsg101 are involved in the sorting of ubiquitinated cell-surface proteins into MVBs by assembling into a so-called ESCRT-I complex that associates transiently with endosomal membranes [57,58]. Ubiquitin-binding domains are found in several other Class E proteins. Vps27 and its mammalian homolog Hrs have two copies of this motif, and mutations in both ubiquitin-binding domains induce the same phenotype as in Vps23/Tsg101 point mutations, namely the formation of an enlarged vacuole [59]. The interaction between Hrs and Tsg101 is controlled by a C-terminal sequence of Tsg101 [60]. The Hrs–Tsg101 complex is required for the recruitment of assembled ESCRT-1 complex to the MVB [61]. Ubiquitinated Hrs, together with ESCRT-I, selects ubiquitinated molecules for entry into internal vesicles of MVBs [62].

Recruitment of ESCRT-I is followed by that of ESCRT-II and ESCRT-III [63,64]. These complexes direct sorted molecules into invaginating vesicles and so allow MVB formation. In yeast, ubiquitin is removed from sorted molecules before entry into the lumenal vesicles of MVBs. The enzymatic activity of the deubiquitinating enzyme Doa4 is required and is recruited after the complete assembly of ESCRT-III [65]. Finally, membrane domains that contain the sorted molecules invaginate and bud as vesicles into the lumen of MVBs. The AAA-ATPase Vps4 or its mammalian homolog, SKD1, both disassembles and releases the entire sorting machinery from MVB membranes. At this point, the ESCRT complexes recycle back into the cytoplasm for sorting of future membrane proteins into MVBs [66]. The full complement of the ESCRT components in mammalian cells and the role of ubiquitin addition and removal in MVB formation remain to be established.

The MVB sorting mechanisms likely differ between the different APCs. Stimulation of DCs with LPS induces the fusion of the internal vesicles of MVBs with the MVB delimiting membrane. The MVBs were proposed to transform into tubular endosomal compartments before transport to the plasma membrane [19,20]. By contrast, in B cells and cytotoxic T cells, the fusion of the MVB with the plasma membrane predominantly leads to release of internal vesicles of MVBs as exosomes [67]. Both exosomes and the internal vesicles of MVBs are enriched in cholesterol, sphingomyelin and components of the machinery that catalyzes MVB formation such as Tsg101 [16,68].

Tetraspanins and glycosylphosphatidylinositol-anchored proteins are known constituents of raft-like microdomains and are present in exosomes and MVBs as well, which suggests that lipid microdomains are involved in sorting of proteins into the internal vesicles in MVBs [69,70].

So far, neither have knockout mice been described for ESCRT-1 in mammalian species, nor has it been possible to examine the effect of dominant-negative mutations, for example in VPS4-SKD1 on Class II trafficking in primary APCs. While it is more likely than not that the ESCRT complex will occupy a central role in trafficking and endosomal localization of Class II MHC molecules, there are at present no experimental data to document its involvement. The extraordinarily dynamic behavior of Class II MHC trafficking and localization, the multiple signals for endosomal delivery and retrieval already demonstrated in the cytoplasmic domains of Class II molecules and Ii will be compared further once the role of ESCRT complexes in these processes is fully understood. An interesting area that merits exploration in the context of Class II trafficking is the unique role played by glycosphingolipids and their metabolites [71].

Tetraspanins and rafts

A search for unknown molecules that might play a role in peptide loading of Class II molecules led to the discovery of tetraspanin family members CD63 and CD82 as interaction partners for Class II MHC products [72,73]. Tetraspanins are molecules that contain four membrane-spanning domains and short cytoplasmic tails. They form homodimers or heterodimers through their stalk subdomain, while their head subdomain appears to serve as a contact site for other membrane proteins, such as integrins [18,74]. Tetraspanins function as the molecular 'glue' that holds together some of these cell-surface-disposed microdomains [74]. In an attempt to identify molecules that associate with Class II MHC molecules, monoclonal antibodies were raised against purified Class II MHC-containing compartments from human B cells and DCs, which yielded the tetraspanins CD82 and CD63 [72,73]. Both CD82 and CD63 associate with endosome-localized Class II MHC before cell-surface expression of Class II MHC. By contrast, in DCs, the tetraspanins CD9, CD53 and CD81 associate with Class II molecules at the cell surface [73]. The contribution of tetraspanins to Class II MHC-restricted antigen presentation, especially related to subcellular localization of Class II MHC molecules is not yet known. Nevertheless, in B cells, CD82 and CD63 form complexes with Class II MHC, H2-DM and H2-DO in endosomal compartments [72], which suggests a role for tetraspan proteins in loading of peptides onto Class II MHC molecules. The contribution of tetraspanin CD81 during conjugate formation of B cells and T cells was shown by use of a CD81–eGFP fusion protein. Upon interaction of antigen-experienced B cells with T cells, CD81–eGFP relocalized to clusters in the central portion of the B-cell–T-cell interface [75]. This is consistent with a role for the tetraspanin CD81 at the cell surface in stimulating the activation of antigen-specific T cells, restricted by Class II MHC.

Localization of Class II MHC in microdomains in human DCs and B cells is readily analyzed through the use of a monoclonal antibody, FN1, that specifically recognizes oligomerized, not monomeric human Class II MHC molecules [76,77]. Tetraspanins are major constituents of FN1-reactive clusters and localize at least in part to cholesterol- and glycosphingolipid-enriched lipid rafts. In B cells, most Class II MHC molecules probably localize constitutively to lipid rafts [33,78]. The distinction between lipid rafts, glycosphingolipid-enriched microdomains and other specializations of the plasma membrane are treated rather loosely by different investigators [79]. Class II MHC molecules in FN-1-positive microdomains show enrichment of antigen-derived peptides, and these microdomains are already formed in late endosomal compartments [17]. The importance for Class II MHC localization to microdomains is also apparent in DCs. When monocyte-derived DCs are treated with LPS, Class II cell-surface expression increases about five-fold, whereas the number of Class II MHC molecules in FN1-positive domains increases ~20-fold [18]. The simultaneous presence of antigenic peptide-loaded Class II MHC complexes and co-stimulatory molecules in microdomains on the surface of APCs is likely to optimize recognition by the T cell receptor. Indeed, the co-stimulatory molecule CD86, strongly upregulated on the surface of DCs upon induction of maturation, segregates into FN-1 microdomains, and their disruption abolishes the ability of DCs to stimulate antigen-specific T cells [17]. In bone-marrow-derived DCs treated with antigen and LPS, endosomal transport vesicles develop that co-express Class II, Class I MHC and CD86, as shown by immunoelectron microscopy. The clustered presence of antigen-derived peptide-loaded Class II, Class I MHC and CD86 persists after their arrival at the cell surface [7]. Taken together, peptide–Class-II complexes are enriched in specific lipid microdomains in B cells and DCs, probably as a means to optimize activation of antigen-specific T cells, as supported by the inhibitory effect on antigen presentation when these domains are disrupted. Indeed, protein reorganization at the interface between the T cell and the APC plays an important role in T cell activation [17,18,33,78].

Domain segregation within endosomes

Class II MHC molecules segregate in microdomains in endosomal compartments as well as on the cell surface [17,19,72,80]. Class II MHC, newly loaded with antigenic peptide, appears enriched in tetraspanin microdomains, whereas empty Class II MHC or pre-existing peptide-loaded Class II MHC can be more evenly distributed in the lipid membrane [17].

When mouse immature bone-marrow-derived DCs are exposed to hen egg lysozyme in the absence of inflammatory agents, intact lysozyme accumulates in H2-M-positive lysosomes, yet no peptide-loaded Class II MHC is detected [6,7,81]. When DC maturation is now induced by addition of LPS, Class II MHC–peptide complexes are formed. Although lysozyme itself remained localized in lysosomal compartments, peptide–Class-II MHC complexes appeared first in transport vesicles and later on the cell surface of mature DCs. These transport vesicles do not contain lysosomal markers such as lysosome-associated membrane

protein (LAMP) and H2-M [7]. Sorting of Class II MHC from other endosomal constituents occurs in activated human DCs as well. Analysis by immuno-electron microscopy of human DCs at an intermediate stage of maturation shows an increase in Class II MHC in small tubules and vesicles, whereas the tetraspanin CD63 and H-2M are retained in multilaminar Class-II-positive endosomal compartments [20]. In contrast to what is found in mouse DCs, immature human DCs already contain most peptides associated with Class II MHC. The peptide repertoire on Class II MHC products was determined in immature and mature DCs by sequence analysis of material from monocyte-derived DCs and human tonsil APCs (H. Kropshofer, pers. commun.).

Transport of peptide-loaded Class II MHC to the cell surface

Immature DCs contain mostly vesicular Class II MHC-positive endosomal compartments, readily visualized in DCs obtained from mice expressing Class-II–eGFP. The dynamic nature of endosomes in bone marrow DCs of mice expressing Class-II–eGFP is captured by confocal microscopy in time-lapse mode (Figure 2). The translocation of eGFP-labeled Class II molecules from late endosomes to the cell surface has been visualized in human melanoma cells [82] and mouse bone-marrow-derived DCs [21,22,83]. LPS-induced maturation of bone-marrow-derived DCs induces the formation of tubular Class-II-positive compartments [22]. These tubular compartments are similar in size to those seen by immuno-electron microscopy in a DC cell line exposed to LPS and might be derived from MVBs [19]. The resolution of present immunofluorescence microscopy techniques does not allow adequate resolution to address this particular question. It might be worthwhile to consider more advanced optical methods, such as 4π microscopy, for three-dimensional imaging at a higher resolution than currently obtainable with common confocal techniques to explore the conversion of MVBs into tubular compartments in living cells [84].

It now appears that a T-cell-mediated signal is required for intracellular transport of Class II MHC to the cell surface [21]. DCs loaded with protein antigen respond to addition of naïve antigen-specific T cells by formation of tubular compartments. T cells with antigen receptors of irrelevant specificity fail to do so. Polarized Class-II-positive tubules that point towards T cells form within minutes, are long (15–20 μm on average), are strictly antigen-dependent and require an intact microtubular apparatus [21] (Figure 3).

It is not sufficient for a DC to be loaded with antigen: an additional microbe-derived signal in the form of a TLR ligand is required to induce T-cell-polarized endosomal tubulation. Myeloid differentiation factor 88 (MyD88) is an adaptor molecule involved in signaling through microbial pattern recognition molecules such as TLRs [4]. In MyD88-deficient Class-II–eGFP-positive DCs, extended Class-II-positive structures fail to form upon exposure to antigen-specific T cells. Accordingly, normal Class-II–eGFP-positive DCs loaded with LPS-free antigen do not support the formation of tubular endosomes, whereas inclusion of LPS to such DCs restores the formation of tubular endosomal compartments [83]. The relationship between LPS-evoked tubules, which are rather short (~5 μm) [19,22], and the longer, T-cell-polarized Class-II-positive endosomal structures is not yet clear. Both types of tubular endosomes likely share a common origin, and the involvement of signaling pathways that act in synergy (T-cell-dependent signals and signals delivered as microbial signatures) might be the explanation.

Concluding remarks

The functional importance of the tubular mode of transport of peptide-loaded Class II MHC to the DC plasma membrane might lie in the formation of microdomains [21,85]. Does the induction of long tubular endosomes in DCs require clustering of proteins in the T cell membrane? A partial reduction of the mobility of membrane proteins, as accomplished by glutaradehyde fixation of T cells, correlates with diminished tubulation. By contrast, robust

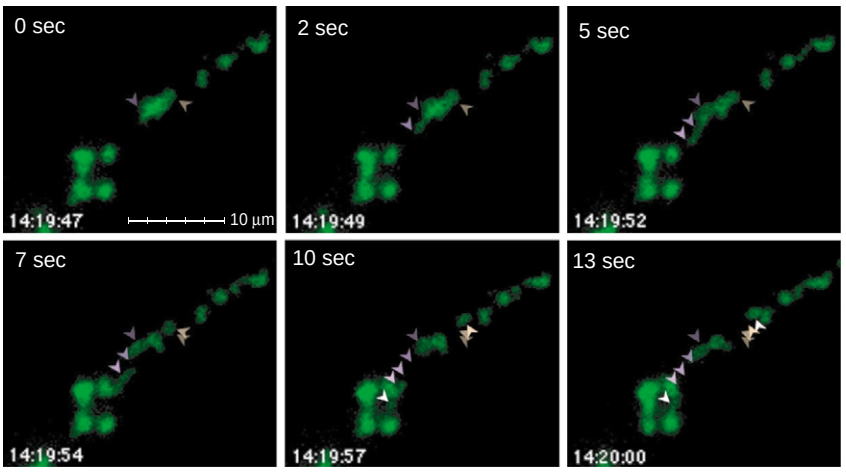

Figure 2. Bidirectional transport of Class-II–eGFP. Bone-marrow-derived dendritic cells were analyzed by time-lapse microscopy in a single optical section. A single dendritic projection of the dendritic cell is shown here, analyzed in time-lapse mode. Bidirectional transport of Class-II–eGFP-positive vesicles is seen. Dark-colored arrowheads represent the starting endosome position, the lighter arrows indicate the progression of the indicated structure. Elapsed time in seconds (upper left corner).

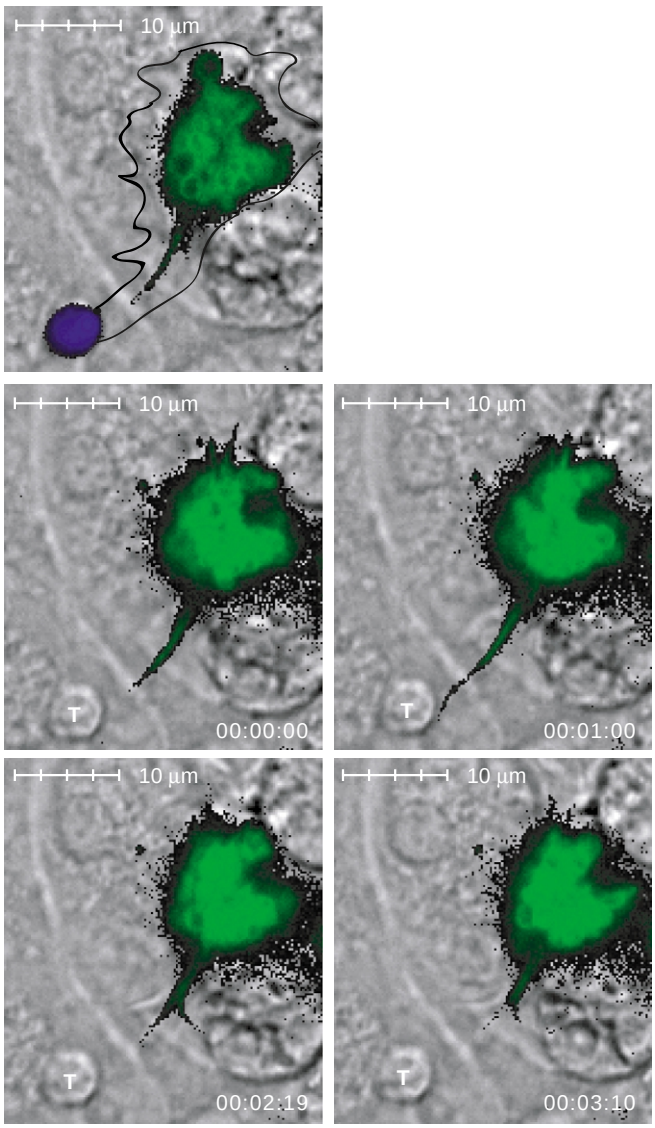

Figure 3. T-cell-polarized Class-II–eGFP-positive tubular endosomes. Bone-marrow-derived dendritic cells (day 4 of culture), cultured on coverslips, were incubated with ovalbumin overnight (200 μM) to induce display of ovalbumin peptide on Class II MHC at the cell membrane. Naïve ovalbumin-specific T cells from OTII transgenic mice were stained with the nuclear dye Hoechst 33258 and brought into contact with dendritic cells by a 30-s centrifugation. Superposition of the bright-field image, green fluorescence (dendritic cells) and blue fluorescence (T cells) is shown. In the absence of ovalbumin, or when T cells of wrong specificity are added, no such tubulation is seen [21].

fixation using paraformaldehyde results in a complete block in T-cell-polarized tubulation [85]. Thus, the rearrangement of proteins on the T cell surface is required to enable antigen-specific T cells to induce tubular endosomes in DCs. The insights gained through a genetic analysis of endocytosis in yeast remain to be extended fully to a study of MVB formation in mammalian cells and more specifically to that of Class-II-restricted antigen presentation. How signals delivered to an APC result in rearrangement of intracellular traffic along the endocytic pathway is still an unanswered question. However, as an experimental model, APCs might be of considerable use in dissection of the mammalian endocytic pathway and its regulation. The combined use of confocal microscopy techniques and biochemical methods in the time-resolved analysis of live cells should help clarify the precise mechanisms of antigen presentation and the initiation of adaptive immune responses.

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