Understanding the cell biology of antigen presentation: the dendritic cell contribution

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The study of the cell biology of antigen processing and presentation has greatly contributed to our understanding of the immune response. The work of many immunologically inclined cell biologists has also permitted us to gain new insights on cellular mechanisms shared by many cell types. Dendritic cells are master regulators of the immune system and consequently have received a lot of attention in recent years. With the aim of controlling antigen processing and presentation, the solutions used by dendritic cells to respond to environmental changes are numerous and surprising. In the presence of pathogens, dendritic cells regulate strongly their endocytic pathway by interfering with uptake, proteolysis, membrane dynamics and transport in and out of the lysosome to become the most potent antigen-presenting cells known.

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Abbreviations

APC	antigen-presenting cell
CIIV	class II vesicle
DC	dendritic cell
iDC	immature DC
Ii	invariant chain
LPS	lipopolysaccharide
MIIC	MHC II compartment
mDC	mature DC
MHC	major histocompatibility complex
MLB	multilamellar body
MVB	multivesicular body
TAP	transporter-associated with antigen presentation

Introduction

Dendritic cells (DCs) play a unique role in the initiation of the immune response, owing to their exceptionally strong capacity for presenting antigens to naive T lymphocytes [1]. DCs migrate to peripheral organs and monitor their environment for the presence of microorganisms. Detection by organ-resident DCs of pathogen-associated molecules such as lipopolysaccharides (LPSs) induces maturation and migration towards the lymph node [2]. Immature DCs (iDCs) are thought to act as sentinels that detect and accumulate foreign antigens, whereas mature DCs (mDCs) present captured antigens to T cells in the lymphoid tissues. In addition, recent observations suggest that DCs might also induce peripheral tolerance by presenting self-antigens to and anergising auto-reactive T cells [3]. DCs are therefore master regulators of the immune system and their maturation reflects an ordered series of signal-dependent events that result in specific alteration of gene expression, intracellular protein targeting and organelle biogenesis, leading to potent immunomodulatory functions.

To trigger an immune response, protein antigens have to be converted to short peptides, loaded onto major histocompatibility complex (MHC) dimers and presented at the surface of antigen-presenting cells (APCs). Peptide–MHC complexes serve as ligands for antigen-specific receptors on T lymphocytes, which are activated with the help of co-stimulatory molecules (such as CD86 or CD40). MHC class I molecules interact mostly with cytosolic self or viral peptides; MHC class II are most often associated with foreign peptides generated in the endocytic pathway. DCs have developed regulation mechanisms that are particularly efficient at influencing the transport and loading of MHC molecules during maturation [4].

We describe here recent and particularly exciting findings on the regulation of antigen presentation during DC activation and their contribution to deciphering lysosomal function.

Keeping control of lysosomes

MHC class II expression is restricted to professional APCs, including B lymphocytes, DCs and macrophages, which are specialised in the stimulation of T cells and the regulation of the immune system. MHC class II molecules, to stimulate CD4+ T cells, must transit through endocytic organelles, before exogenous peptide loading and cell surface arrival [5]. Antigen processing is totally dependent on endosomal proteolysis to generate the peptides loaded onto MHC molecules [6]. Interestingly, MHC class II molecules are even further dependent on proteolysis, owing to their association with invariant chain (Ii) [7,8]. Ii is required for MHC II endosomal targeting and strerically prevents the binding of exogenous peptides to MHC dimers [8]. Endosomal MHC-class-II–Ii complexes must therefore be rendered competent for

antigenic peptide loading by degrading Ii, which is cleaved sequentially and accumulates in discrete intermediates still associated with MHC II, known as p22 (LIP [leupeptin-induced protein], 22 kDa), p10 (SLIP [small LIP], 10 kDa) and, lastly, CLIP (class II-associated Ii peptide) [7]. CLIP–MHC-II complexes are the targets of H2-DM and H2-DO, which are both heterodimeric molecules homologous to MHC II and which catalyse the removal of the Ii peptide from the class II groove and favour its replacement with a different antigenic peptide [9].

Increasing evidence suggest that DCs can modulate their proteolytic activity to control antigen presentation. Developmental control of proteolysis was first shown in mouse DCs, in which Ii degradation is modulated after LPS or cytokine stimulation [10,11•,12]. Interestingly, cathepsin S, an APC-specific endoprotease responsible for p10 degradation, is inhibited in iDCs [10]. Cystatin C, a natural inhibitor of cysteine proteases, was proposed to play an active role in this process by being targeted to the lysosomes of iDCs but not to those of mDCs [10]. A relatively slow rate of Ii degradation in iDCs is likely to favour the coordinated transport and retention of MHC II molecules in the lysosomes of these cells. This observation has now been extended to show that a general activation of endosomal proteolysis occurs during DC maturation [13••]. Maturation induces vacuolar proton pump activation, thus leading to enhanced lysosomal acidification and facilitating efficient formation of peptide–MHC complexes. This finding explains the relative stability of internalised antigens and the inefficiency of MHC II–peptide loading in iDCs [14]. The inducible recruitment by endosomal membranes of the V1 sector of the proton pump decreases the lysosomal pH by 1 unit (to pH 4.5) and favours the active conversion of lysosomal proteases and enzymes involved in antigen presentation, such as the γ-interferon-inducible lysosomal thiol reductase (GILT) [15].

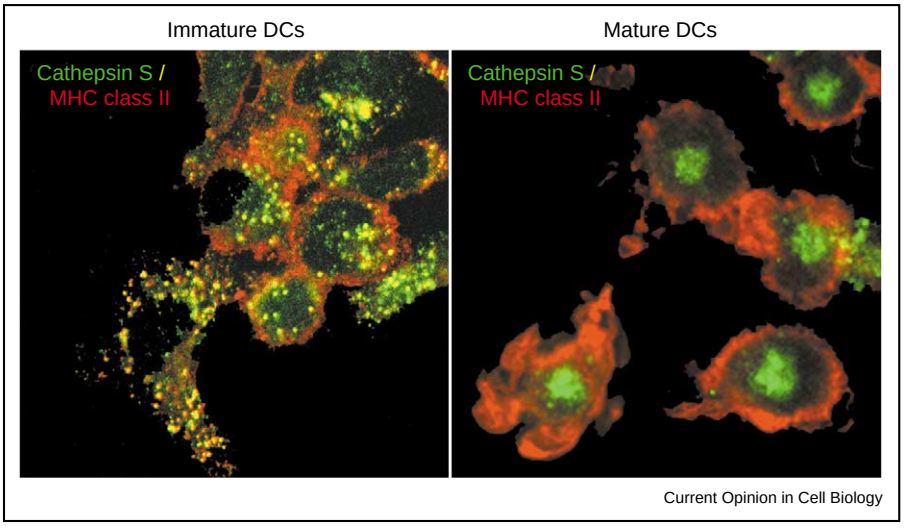
Although very appealing, this model has still several caveats. For instance, the fact that MHC class II half-life is relatively short in iDCs [16–18] should be reconciled with the mediocre proteolytic activity of these cells. In addition, cathepsin S, well known to be also active at neutral pH [19], should not be sensitive to acidification. Natural protease inhibitors (such as cystatins) and potential intraendosomal segregation could therefore also contribute to the regulation of proteolysis [12] as well as other mechanisms.

Invariant chain, although a proteolytic target, is paradoxically directly involved in the control of lysosomal degradation. Ii exists as two alternatively spliced forms, p31 and p41, distinguished by an insert of a 64-amino-acid domain in the lumenal portion of p41 [8]. Both *in vitro* and *in vivo*, p41 has been shown to inhibit the major lysosomal cysteine protease cathepsin L and to provide better lysosomal targeting and stability to this enzyme [20,21•,22,23]. Surprisingly and independently of p41 function, the absence of Ii favours H2-DM degradation in mDCs, revealing a complex interplay, between the presentation machinery and the lysosomal environment [24]. The complexity of this regulation is further demonstrated by the observation that cathepsin L activity is completely inhibited in mouse DCs, even when overexpressed by transgenesis [25•]. The lysosomal environment of DCs is therefore regulated by multiple factors having a broad or restricted specificity and could serve as a model to understand how cells control lysosomal activity during their development.

A way out of the lysosomes

One of the most impressive features of DC maturation is the redistribution of MHC II molecules from intracellular lysosomal compartments to the surface of mature cells [17] (Figure 1). The developmental activation of MHC class II transport is specific to DCs and has been compared with a gearbox, through which immature 'idling' DCs 'gear up' their antigen-presenting activity. The observation that lysosomes in DCs can export their contents to the cell surface in a regulated fashion has reconciled divergent observations describing the identity of the organelles involved in MHC II loading and export. The detection over time of several MHC-II-containing compartments in maturing primary DCs has confirmed what had been observed in different transformed B cell lines and debated over the years [26]. MHC II molecules on their way to the surface can traffic through endocytic compartments both containing lysosomal markers (MIIC [MHC II compartment]) or not (CIIV [class II vesicles]) [17]. In addition, the understanding that multivesicular body (MVB) and multilamellar body (MLB) MHC-II-containing compartments represent conventional late endosomes and lysosomes, and not specialised compartments, has been an important step in this process [27]. Lysosomes have long been considered as a 'cul-de-sac' for endocytic transport, where internalised material is degraded by multiple hydrolysing enzymes. The finding that DC lysosomes can initiate a previously unknown exit pathway, by which selected molecules can be rescued from degradation, introduces a totally new concept (Figure 2). Electron microscopy and video imaging has revealed that lysosomes undergo a full redistribution of their internal membrane contents, leading to lysosomal proteins sorting, tubule formation and transport towards the cell surface [28••,29•,30••,31••]. In iDCs, MHC II is targeted to the internal vesicles of MVBs and segregated from the peptide-editor H2-DM, which localises in the peripheral membrane of the organelles [28••]. Upon activation, intermixing of the membranes allows contact of MHC II with other lysosomal molecules, while tubule formation promotes its sorting. This exit pathway is inducible,

Figure 1



Confocal microscopy of immature and mature DCs stained for MHC class II (red) and cathepsin S (green). Cathepsin S is mainly localised in the lysosomes and MHC class II is redistributed to the surface of DCs upon LPS stimulation.

although it is clearly different from the previously described exocytosis of secretory lysosomes observed in cytotoxic T cells [32] or the release of MHC-containing exosomes by B cells [33].

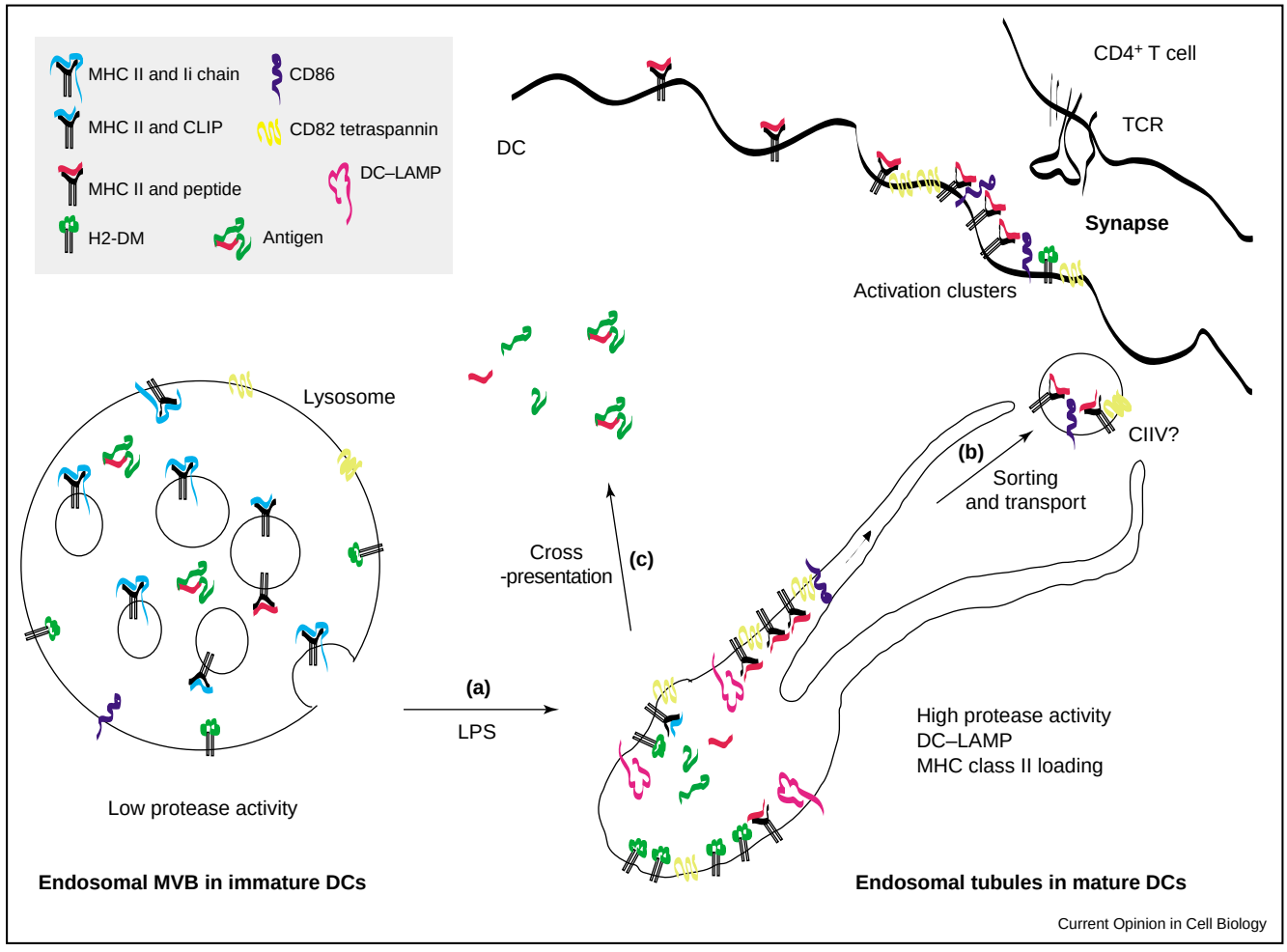
Exosomes are likely to be the result of a direct fusion of endosomal MVBs with the plasma membrane, causing the release of their internal membranes in the extracellular media [34]. DCs, like B cells, secrete exosomes; however, perhaps because of tubule formation, secretion is reduced during DC maturation and contributes only partially (10%) to the total MHC II delivery to the surface [34]. The tubulo-vesicularisation of MVBs and MLBs upon DC activation demonstrate that lysosomes are not dead ends for membrane proteins; it also explains the previous characterisation in these cells of CIIVs [17], which are likely to represent a purified tubule population. However, the mechanism by which sorting of MHC II from other lysosomal-resident molecules (such as H2-DM or LAMP-2 [lysosome-associated membrane protein 2]) is achieved, and the fate of soluble internalised material, still have to be investigated [30••]. DC-specific molecules such as DC–LAMP [35,29•], which is induced and targeted both to lysosomes and tubules during maturation, could play a key role in this event.

Invariant chain could also participate in the intravesicular sorting of MHC II. The potential role of ubiquitination, now known to contribute actively to the formation of MVBs [36], should also be evaluated in iDCs. Lysosomes have been shown to form tubular extension in macrophages and B cells but only to facilitate phagosome–lysosome fusion and not surface delivery [37].

Whether the tubule-mediated pathway to the surface is unique to DCs remains to be examined. Clearly some differences observed among species and the involvement of known or DC-specific members of the coat, Rabs and SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein receptor)-protein families, will have to be clarified.

The formation of the immunological synapse containing various proteins and lipids required for efficient T cell activation is necessary to maximise antigen presentation and its immune consequences [38]. The possibility that the endosomal exit pathway contributes to the establishment of the synapse between DCs and T cells is currently under investigation. This concept is strongly supported by the observations that co-stimulatory molecules (CD86) are clustered with MHC II molecules en route for the surface and that T cells engagement polarises the direction of tubules formation [14,31••]. Targeted delivery of pre-assembled presentation molecules would greatly facilitate the scanning by T cell receptors of the DC surface. The rapid detection of matching peptide–MHC complexes and activation molecules is likely to be an important step in optimising synapse formation and DC–T-cell interaction. Internal membrane organisation and lipid dynamics are therefore vital in regulating antigen presentation. This view is reinforced by the involvement of membrane microdomains in synapse formation and T cell activation. As much as 50% of surface MHC class II has been shown to reside in lipid rafts, and the disruption of these cholesterol-enriched microdomains inhibits antigen presentation [39,40].

Figure 2



In iDCs, MHC class II molecules are sorted away from H2-DM in the internal vesicles of MVBs. The combination of low endosomal proteolytic activity and MHC class II segregation in lysosomes strongly decrease the efficiency of antigenic peptide loading in iDCs. **(a)** Stimulation of DCs by pathogen motifs, such as LPS, induces lysosomal membrane redistribution, proteolytic activation and upregulation of specific molecules by the maturing cells. **(b)** These events induce the sorting of peptide-loaded MHC class II into a tubule–vesicular membrane network before their arrival at the cell surface. It is still debated if this step occurs directly by fusion of the tubules with the plasma membrane or an additional vesicular step is required (CIIV). This last triage step permits the pre-assembly of discrete stimulation packages comprising MHC II molecules, B7-2 and probably other co-stimulatory molecules. This assembly contributes directly to immunological synapse formation by polarising the delivery of all the necessary antigen presentation components towards the T cell contact zone. **(c)** The passage of antigens from the endocytic pathway towards the cytosol permits MHC class I presentation of peptides from exogenous origin (cross-presentation). This phenomenon is specific to DCs and is induced by interactions with pathogen motifs or helper T cells; however, the molecular machinery implicated in this process is still elusive.

Interestingly, clustering of functional peptide-loaded MHC II molecules (5–20%) and CD86 within tetraspannins (e.g. CD82)-containing microdomains has been detected not only on the cell surface [41••], but also in lysosomes, where all these molecules are enriched [42,43]. Although contradictory on the importance of cholesterol-enriched microdomains in antigen presentation, these reports have both introduced the concept that all MHC II molecules are not functionally equal and that pre-assembled endosomal clusters targeted to the surface might have specific functions. In addition to the facilitation of T cell activation by recruitment of MHC-II-enriched microdomains at the synapse, a function of signal transduction within DCs has also been proposed for these clustered MHC II molecules [44•]. This signalling pathway would be used by DCs to coordinate MHC-class-I-restricted presentation of exogenous antigens (cross-presentation) with MHC class II presentation [44•]. This DC-specific function is likely to increase dramatically the spectrum and therefore the efficiency of the immune response. Conceptually this type of coordination might offer an interesting read-out to understand the general role of membrane microdomains in signal transduction.

Another way out of the lysosomes?

MHC class I peptide loading occurs within the ER. The quasi-exclusive participation of the proteasome for antigenic peptide generation and of TAP (transporter-associated with antigen presentation) transporters for cytosol-to-ER translocation is now well documented [45,46]. Only DCs, among other APCs, are capable of performing cross-presentation,which consists of the loading by MHC class I of exogenous peptides derived from endocytosed antigens [47,48••]. Cross-presentation implies the possibility for antigens to exit lysosomes or alternatively for exogenous peptides to be loaded onto endosomal MHC class I which are abundant in DCs [49]. Cross-presentation requires proteasome activity and functional TAP molecules [47]. This strict dependency supports the model that lysosome-to-cytosol translocation of the internalised antigens has to occur before proteasome processing, TAP-mediated transport in the ER lumen and MHC loading [47]. Lysosome-to-cytosol passage seems to be size-dependent and controlled by DC maturation and T cell interaction [44•].

Recent evidence on the recruitment of ER membranes during phagosome formation has opened new possibilities to explain the mechanism of cross-presentation [50••]. By acquiring ER membranes, phagosomes also receive TAP transporters, as well as associated chaperones, proteasome and translocon units. TAP molecules could still be functional after incorporation in phagosome membranes and promote the export of exogenous antigens from phagolysosomes to the cytosol. Thus the need for a specialised transport machinery would be eliminated. Alternatively, TAP molecules and the proteasome could play a role, direct or indirect, in ER–phagosomes fusion. Their implication in cross-presentation could therefore be a side effect of this role and consequently MHC class I loading with exogenous antigens could occur directly in the endocytic pathway [49]. The clear identification of the molecules responsible for this phenomenon will probably solve this traffic dilemma, which represents a fascinating cell biology problem.

Conclusions

In addition to being important for induction of immune responses, DCs offer multiple examples of specialised regulation of their endosomal system. The existence of these processes implies that multiple cell types could use the same processes for different purposes. By emphasising one or more of these processes normally difficult to detect in standard laboratory cell lines, specialised cells allow their identification and study. DCs are an incredible source of such membrane-traffic processes, still to be fully understood.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S: **Antigen presentation and T cell stimulation by dendritic cells**. *Annu Rev Immunol* 2002, **20**:621-667.

2. Kaisho T, Akira S: **Dendritic-cell function in Toll-like receptor- and MyD88-knockout mice**. *Trends Immunol* 2001, **22**:78-83.

3. Steinman RM, Nussenzweig MC: **Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance**. *Proc Natl Acad Sci USA* 2002, **99**:351-358.

4. Mellman I, Steinman RM: **Dendritic cells: specialized and regulated antigen processing machines**. *Cell* 2001, **106**:255-258.

5. Hiltbold EM, Roche PA: **Trafficking of MHC class II molecules in the late secretory pathway**. *Curr Opin Immunol* 2002, **14**:30-35.

6. Watts C: **Antigen processing in the endocytic compartment**. *Curr Opin Immunol* 2001, **13**:26-31.

7. Riese RJ, Chapman H: **Cathepsins and compartimentalization in antigen presentation**. *Curr Opin Immunol* 2000, **12**:107-113.

8. Lennon-Dumenil AM, Bakker AH, Wolf-Bryant P, Ploegh HL, Lagaudriere-Gesbert C: **A closer look at proteolysis and MHC-class-II-restricted antigen presentation**. *Curr Opin Immunol* 2002, **14**:15-21.

9. Alfonso C, Karlson L: **Nonclassical MHC class II molecules**. *Annu Rev Immunol* 2000, **18**:113-142.

10. Pierre P, Mellman I: **Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells**. *Cell* 1998, **93**:1135-1145.

11. • Fiebiger E, Meraner P, Weber E, Fang IF, Stingl G, Ploegh H, Maurer D: **Cytokines regulate proteolysis in major histocompatibility complex class II-dependent antigen presentation by dendritic cells**. *J Exp Med* 2001, **193**:881-892.
This demonstrates that cytokines can regulate proteolysis in dendritic cells.

12. Lautwein A, Burster T, Lennon-Dumenil AM, Overkleeft HS, Weber E, Kalbacher H, Driessen C: **Inflammatory stimuli recruit cathepsin activity to late endosomal compartments in human dendritic cells**. *Eur J Immunol* 2002, **32**:3348-3357.

13. •• Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I: **Activation of lysosomal function during dendritic cell maturation**. *Science* 2003, **299**:1400-1403.
This shows that dendritic cells regulate endosomal pH and protease activity by inducible recruitment of the vacuolar proton pump.

14. Turley S, Inaba K, Garret WS, Ebersold M, Unternaehrer J, Steinman RM, Mellman I: **Transport of peptide-MHC class II complexes in developing dendritic cells**. *Science* 2000, **288**:522-527.

15. Honey K, Duff M, Beers C, Brissette WH, Elliott EA, Peters C, Maric M, Cresswell P, Rudensky A: **Cathepsin S regulates the expression of cathepsin L and the turnover of gamma-interferon-inducible lysosomal thiol reductase in B lymphocytes**. *J Biol Chem* 2001, **276**:22573-22578.

16. Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A: **Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells**. *Nature* 1997, **388**:782-787.

17. Pierre P, Turley SJ, Gatti E, Hull M, Melzer J, Mirza A, Inaba K, Steinman RM, Mellman I: **Developmental regulation of MHC class II transport in mouse dendritic cells**. *Nature* 1997, **388**:787-792.

18. Villadangos JA, Cardoso M, Steptoe RJ, van Berkel D, Pooley J, Carbone FR, Shortman K: **MHC class II expression is regulated in dendritic cells independently of invariant chain degradation**. *Immunity* 2001, **14**:739-749.

19. Kirschke H, Wiederanders B: **Cathepsin S and related lysosomal endopeptidases**. *Methods Enzymol* 1994, **244**:500-511.

20. Fineschi B, Arneson LS, Naujokas MF, Miller J: **Proteolysis of major histocompatibility complex class II-associated invariant chain is regulated by the alternatively spliced gene product p41**. *Proc Natl Acad Sci USA* 1995, **92**:10257-10261.

21. • Lennon-Dumenil AM, Roberts RA, Valentijn K, Driessen C, Overkleeft HS, Erickson A, Peters PJ, Bikoff E, Ploegh HL, Wolf Bryant P: **The p41 isoform of invariant chain is a chaperone for cathepsin L**. *EMBO J* 2001, **20**:4055-4064.
This shows that p41 facilitates cat L sorting and survival in lysosomes.

22. Bevec T, Stoka V, Pungercic G, Dolenc I, Turk V: **Major Histocompatibility complex class II-associated p41 invariant chain fragment is a strong inhibitor of lysosomal cathepsin L**. *J Exp Med* 1996, **183**:1331-1338.

23. Fiebiger E, Maehr R, Villadangos J, Weber E, Erickson A, Bikoff E, Ploegh HL, Lennon-Dumenil AM: **Invariant chain controls the activity of extracellular cathepsin L**. *J Exp Med* 2002, **196**:1263-1269.

24. Pierre P, Shachar I, Matza D, Gatti E, Flavell R, Mellman I: **Invariant chain controls H2-M proteolysis in mouse splenocytes and dendritic cells**. *J Exp Med* 2000, **191**:1057-1062.

25. • Beers C, Honey K, Fink S, Forbush K, Rudensky A: **Differential regulation of cathepsin S and cathepsin L in interferon gamma-treated macrophages**. *J Exp Med* 2003, **197**:169-179.
This shows, among other things, that Cat L activity is inhibited in dendritic cells, despite high levels of expression.

26. Pierre P, Mellman I: **Exploring the mechanisms of antigen processing by cell fractionation**. *Curr Opin Immunol* 1998, **10**:145-153.

27. Kleijmeer MJ, Morkowski S, Griffith JM, Rudensky AY, Geuze HJ: **Major histocompatibility complex class II compartments in human and mouse B lymphoblasts represent conventional endocytic compartments**. *J Cell Biol* 1997, **139**:639-649.

28. •• Kleijmeer M, Ramm G, Schuurhuis D, Griffith J, Rescigno M, Ricciardi-Castagnoli P, Rudensky AY, Ossendorp F, Melief CJ, Stoorvogel W *et al.*: **Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells**. *J Cell Biol* 2001, **155**:53-63.
This paper first demonstrates tubule formation and MHC class II sorting in multivesicullar bodies.

29. • Barois N, De Saint-Vis B, Lebecque S, Geuze HJ, Kleijmeer MJ: **MHC class II compartments in human dendritic cells undergo profound structural changes upon activation**. *Traffic* 2002, **3**:894-905.
This paper first demonstrates tubule formation and MHC class II sorting in human cells.

30. •• Chow A, Toomre D, Garrett W, Mellman I: **Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane**. *Nature* 2002, **418**:988-994.
The authors demonstrate live lysosome tubules and MHC class II transport to the surface from the lysosomes.

31. •• Boes M, Cerny J, Massol R, Op den Brouw M, Kirchhausen T, Chen J, Ploegh HL: **T-cell engagement of dendritic cells rapidly rearranges MHC class II transport**. *Nature* 2002, **418**:983-988.
This is a back to back paper with Chow *et al.* (2002) [30••]. The authors also demonstrate the role of T cells in polarising the tubules.

32. Blott EJ, Griffiths GM: **Secretory lysosomes**. *Nat Rev Mol Cell Biol* 2002, **3**:122-131.

33. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ: **B lymphocytes secrete antigen-presenting vesicles**. *J Exp Med* 1996, **183**:1161-1172.

34. Thery C, Zitvogel L, Amigorena S: **Exosomes: composition, biogenesis and function**. *Nat Rev Immunol* 2002, **2**:569-579.

35. de Saint-Vis B, Vincent J, Vandenabeele S, Vanbervliet B, Pin JJ, Aït-Yahia S, Patel S, Mattei MG, Banchereau J, Zurawski S *et al.*: **A novel lysosome-associated membrane glycoprotein, DC–LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment**. *Immunity* 1998, **9**:325-336.

36. Katzmann DJ, Babst M, Emr SD: **Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I**. *Cell* 2001, **106**:145-155.

37. Lankar D, Vincent-Schneider H, Briken V, Yokozeki T, Raposo G, Bonnerot C: **Dynamics of major histocompatibility complex class II compartments during B cell receptor-mediated cell activation**. *J Exp Med* 2002, **195**:461-472.

38. Davis DM: **Assembly of the immunological synapse for T cells and NK cells**. *Trends Immunol* 2002, **23**:356-363.

39. Anderson HA, Hiltbold EM, Roche PA: **Concentration of MHC class II molecules in lipid rafts facilitates antigen presentation**. *Nat Immun* 2000, **1**:156-162.

40. Hiltbold EM, Poloso NJ, Roche PA: **MHC class II-peptide complexes and APC lipid rafts accumulate at the immunological synapse**. *J Immunol* 2003, **170**:1329-1338.

41. •• Kropshofer H, Spindeldreher S, Rohn TA, Platania N, Grygar C, Daniel N, Wolpl A, Langen H, Horejsi V, Vogt AB: **Tetraspan microdomains distinct from lipid rafts enrich select peptide–MHC class II complexes**. *Nat Immun* 2002, **3**:61-68.
The data presented in this paper argue against the implication of cholesterol-enriched rafts in MHC II presentation and promote the importance of tetraspannin-enriched domains. Conversely Anderson *et al.* (2000) [39] have demonstrated the importance of lipid rafts in this process.

42. Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ: **Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes**. *J Biol Chem* 1998, **273**:20121-20127.

43. Hammond C, Denzin LK, Pan M, Griffith JM, Geuze HJ, Cresswell P: **The tetraspan protein CD82 is a resident of MHC class II compartments where it associates with HLA-DR, -DM, and -DO molecules**. *J Immunol* 1998, **161**:3282-3291.

44. • Machy P, Serre K, Baillet M, Leserman L: **Induction of MHC class I presentation of exogenous antigen by dendritic cells is controlled by CD4+ T cells engaging class II molecules in cholesterol-rich domains**. *J Immunol* 2002, **168**:1172-1180.
This paper demonstrates a function for MHC class II signalling in cross-presentation by dendritic cells.

45. Kessler B, Hong X, Petrovic J, Borodovsky A, Dantuma NP, Bogyo M, Overkleeft HS, Ploegh H, Glas R: **Pathways accessory to proteasomal proteolysis are less efficient in MHC class I antigen production**. *J Biol Chem* 2002, **278**:10013-10021.

46. Yewdell JW, Bennink JR: **Cut and trim: generating MHC class I peptide ligands**. *Curr Opin Immunol* 2001, **13**:13-18.

47. Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S: **Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells**. *Nat Cell Biol* 1999, **1**:362-368.

48. •• Jung S, Unutmaz D, Wong P, Sano G, De los Santos K, Sparwasser T, Wu S, Vuthoori S, Ko K, Zavala F *et al.*: ***In vivo* depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens**. *Immunity* 2002, **17**:211-220.
The authors used gene targeting and toxin injection to abrogate dendritic cells in mice — a technological 'must see'.

49. Kleijmeer MJ, Escola JM, UytdeHaag FG, Jakobson E, Griffith JM, Osterhaus AD, Stoorvogel W, Melief CJ, Rabouille C, Geuze HJ: **Antigen loading of MHC class I molecules in the endocytic tract**. *Traffic* 2001, **2**:124-137.

50. •• Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, Paiement J, Bergeron JJ, Desjardins M: **Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages**. *Cell* 2002, **110**:119-131.
This paper is the follow-up to the phagosome proteome analysis [51] demonstrating ER membrane recruitment during phagosome formation.

51. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M: **The phagosome proteome: insight into phagosome functions**. *J Cell Biol* 2001, **152**:165-180.