Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell?

Taku Kambayashi1 and Terri M. Laufer2

Abstract | Dendritic cells, macrophages and B cells are regarded as the classical antigen-presenting cells of the immune system. However, in recent years, there has been a rapid increase in the number of cell types that are suggested to present antigens on MHC class II molecules to CD4+ T cells. In this Review, we describe the key characteristics that define an antigen-presenting cell by examining the functions of dendritic cells. We then examine the functions of the haematopoietic cells and non-haematopoietic cells that can express MHC class II molecules and that have been suggested to represent ‘atypical’ antigen-presenting cells. We consider whether any of these cell populations can prime naive CD4+ T cells and, if not, question the effects that they do have on the development of immune responses.

Dendritic cells (DCs), B cells and macrophages constitutively express MHC class II molecules and are regarded as the ‘professional’ antigen-presenting cells (APCs) of the immune system [FIG. 1]. However, the universe of APCs, seemingly so well established, has recently been changing as more and more cell types are proposed to express MHC class II molecules and have antigen-presenting functions [FIG. 1; TABLE 1]. A number of haematopoietic cell types have been suggested to present antigens on MHC class II molecules to CD4+ T cells, including mast cells, basophils, eosinophils, neutrophils, innate lymphoid cells (ILCs) and CD4+ T cells themselves. In addition, MHC class II expression has been detected on non-haematopoietic cell types in the periphery, such as endothelial cells, epithelial cells and lymph node stromal cells (LNSCs). MHC class II-expressing cells also support thymocyte development and tolerance induction in the thymus. However, for the purposes of this article, we focus on how atypical MHC class II+ cells interact with mature CD4+ T cells that have exited the thymus. In this Review, we delineate the qualities that define a true APC and then examine whether any of the atypical MHC class II+ cell populations have these functional capabilities.

Classical APC characteristics

A consideration of the properties of DCs that facilitate the activation of naive CD4+ T cells provides a comprehensive list of characteristics that a bona fide APC should have (BOX 1). Specifically, in addition to their antigen processing and presentation capabilities, and their expression of co-stimulatory molecules, DCs express pattern recognition receptors (PRRs) that mediate activation in response to pathogens. Following PRR-mediated maturation, DCs can migrate to the lymph nodes to promote the activation of naive T cells. Indeed, DCs have been shown to be both necessary and sufficient for the activation of naive T cells1,2. The APC that initiates an immune response must make multiple decisions — for example, whether to actively respond to or tolerate the antigenic challenge, and what type of active immune response to induce. DCs clearly predominate in the induction of T cell proliferation. Interestingly, however, DCs are not required for the development of CD4+ T cell responses under certain conditions3,4, suggesting that other APCs may be able to replace the function of DCs. Thus, we consider whether any non-classical APC can prime naive CD4+ T cells, and discuss the roles that these cells may have in modulating DC-dependent and DC-independent immune responses.

Mast cells as APCs

Mast cells are tissue-resident innate immune cells that are strategically localized at mucosal and submucosal sites in close proximity to the external environment. They have established roles in allergic disease. Following the activation of high-affinity Fc receptor for IgE (FceRI), mast cells degranulate and release pre-stored immunomodulatory molecules — such as tumour necrosis factor...
(TNF) and histamine — which can promote T cell activation both directly and indirectly, through stimulation of APCs. Furthermore, mast cell-deficient mice have defective CD4+ T cell responses in experimental autoimmune encephalomyelitis and in Leishmania major infection, suggesting that CD4+ T cell responses could be altered in the absence of mast cells.

Expression of MHC class II molecules by mast cells. Mast cells can directly present antigens to T cells. Both rodent and human mast cells have been reported to constitutively express MHC class II molecules. The expression of MHC class II by mast cells correlated with their ability to present antigen in vitro to naive CD4+ T cells and to T cell hybridomas. Cytokines such as interleukin-4 (IL-4), interferon-γ (IFNγ) and granulocyte–macrophage colony-stimulating factor (GM-CSF; also known as CSF2) further enhanced the APC function of mast cells. However, these reports were contradicted in follow-up studies by the same group showing that the activation of antigen-specific T cells still occurred when the T cells were cultured with MHC-mismatched mast cells. Moreover, co-culture of mast cells with splenocytes resulted in antigen-independent activation of T cells, which was probably mediated by immunologically active exosomes released by mast cells (BOX 2). These results were consistent with subsequent studies showing that MHC class II molecules mainly reside in intracellular lysosomal compartiments of mast cells, rather than at the cell surface.

More recently, several groups have reported that resting or FcεRI-activated mast cells do not express MHC class II molecules, either on the cell surface or intracellularly. The apparent discrepancies between the earlier and more recent studies may reflect the different protocols that have been used to generate mast cells. The earlier studies generated mast cells from short-term bone marrow cultures (~3 weeks) that were supplemented with IL-3 alone. By contrast, later studies used long-term bone marrow cultures that were supplemented with both IL-3 and stem cell factor (SCF; also known as KIT ligand). Many of the cells derived from the short-term cultures expressed FcεRI but lacked the mast/stem cell growth factor receptor KIT, suggesting that these cultures also contained non-mast cells, including basophils and mast cells from bone marrow cultures less than 3 weeks old that were able to present antigen to T cells.

Although resting bone marrow-derived mast cells from long-term cultures do not constitutively express MHC class II, its expression can be induced on these cells. After activation of bone marrow-derived mast cells with Toll-like receptor 4 (TLR4) agonists and, to a lesser extent, TLR2 agonists in the presence of IFNγ, a large proportion of cells expressed MHC class II and other molecules that are associated with antigen presentation — namely, MHC class II transactivator (CIITA), the invariant chain (II) and H2-DM. Similarly, although freshly isolated mast cells from the peritoneal cavity lacked MHC class II expression, these mast cells expressed MHC class II after in vitro treatment with IL-4 and IFNγ. MHC class II+ mast cells were also found in lymph node sinuses; treatment of...
Table 1 | Features of ‘atypical’ antigen-presenting cells

<table>
<thead>
<tr>
<th>Atypical APC</th>
<th>Features of atypical APC</th>
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<tr>
<td>Mast cells</td>
<td>Ability to promote T cell activation</td>
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<tr>
<td></td>
<td>Naive T cells</td>
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<td>Basophils</td>
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<td>Eosinophils</td>
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<td>Neutrophils</td>
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<tr>
<td>ILC2s</td>
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<td>ILC3s</td>
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<tr>
<td>CD4+ T cells</td>
<td>−</td>
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<tr>
<td>LNSCs</td>
<td>Tolerance induction</td>
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<td>Epithelial cells</td>
<td>Tolerance induction</td>
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Expression of co-stimulatory molecules:
- +/− (CD80 and CD86)
- + (mouse)
- − (human)
- CD80 and CD86

Lymph node migration by APC:
- +

Factors that induce expression of MHC class II on APC:
- TLR agonists, IFNγ, GM-CSF, IL-4 and Notch
- FcεRI, IL-3 and papain
- IL-3, IFNγ and GM-CSF
- Co-culture with T cells, GM-CSF and IFNγ

Expression of other MHC class II-related genes:
- NA
- Unknown
- TLR agonists and IFNγ
- IFNγ
- IFNγ and microbial stimuli

APC, antigen-presenting cell; CD137L, CD137 ligand (also known as TNFSF9); FcεRI, high-affinity Fc receptor for IgE; GM-CSF, granulocyte–macrophage colony-stimulatory factor; ICOSL, ICOS ligand; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; LNSC, lymph node stromal cell; NA, not applicable; OX40L, OX40 ligand (also known as TNFSF4); TCR, T cell receptor; TLR, Toll-like receptor.

mice with lipopolysaccharide or infection with L. major increased the number of mast cells in the draining lymph nodes and further upregulated MHC class II expression. The apparent constitutive expression of MHC class II by lymph node mast cells may be owing to the fact that these cells have matured and migrated following their activation in the tissue. Alternatively, endogenous ligands that induce the expression of MHC class II on mast cells may exist at specific anatomical sites. The latter hypothesis is supported by data identifying Notch–Delta-like protein 1 interactions as an inducer of mast cell MHC class II expression. The requirement for IFNγ and either TLR-mediated or Notch-mediated signalling for MHC class II expression by mast cells may be related to the roles of these pathways in inducing the transcription factor PU.1, which regulates the CIITA promoter.

Functions of MHC class II on mast cells. After MHC class II was expressed by activated bone marrow-derived mast cells, they could process and present soluble protein antigens to previously activated CD4+ T cells in vitro, but they could not prime naive T cells, perhaps because they lacked co-stimulatory molecule expression. However, given that lymph node-resident and peritoneal mast cells that have been activated by IL-4 and IFNγ express both CD80 and CD86 (REFS 20,27), it is possible that some mast cells can present antigens to naive T cells in vivo. In addition to activated T cells, mast cells appear to preferentially expand antigen-specific regulatory T (Treg) cells rather than naive T cells. The activation of Treg cells by MHC class II+ mast cells may contribute to the protective effects of mast cells in mediating tolerance to skin allografts and in preventing nephrotoxic serum nephritis, which are processes that were proposed to involve IL-9 production by Treg cells to recruit mast cells to the graft or injury site. It has been shown that endogenous proteins are effectively presented on MHC class II molecules of mast cells and as such, many of the peptides presented by mast cell MHC class II may be self antigens that stimulate Treg cells.

Does IgE support mast cell antigen-presenting functions? Antigen-specific IgE molecules enhance APC function in a subset of human DCs that express FcεRI by shuttling incorporated antigens to the MHC class II pathway. FcεRI could also potentially facilitate the uptake of antigens into mast cells for more efficient antigen presentation. Indeed, large particulate antigens were taken up by mast cells through an IgE-dependent pathway and presented the antigens to T cells in vitro and in vivo. Moreover, earlier studies using cultured mast cells demonstrated that the uptake of antigens through antigen-specific IgE molecules enhances the T cell-stimulating capacity of mast cells. However, in the latter scenario, the activation of T cells was not mediated through direct antigen presentation by mast cells, as the mast cells used in these experiments failed to express MHC class II. Rather, mast cells transferred FcεRI-incorporated antigens to DCs that secondarily presented the antigens to T cells. Antigens incorporated by mast cells through FcεRI were found to colocalize with secretory compartments, and were released by the mast cells upon reactivation. Thus, although FcεRI may transport antigens to the MHC class II pathway under specific conditions, mast cells that...
Although basophil-derived IL-12, IL-18, and IL-23, whether they truly present antigen, are produced by professional APCs, such as DCs, is still necessary for the differentiation of T helper 2 (T₂) cells. Indeed, a number of studies have suggested that basophil-derived IL-4 is crucial for the development of T₂ cell responses to cytokine proteases, allergens and extracellular parasites.

**Basophils modulate T cell responses**

Basophils are a rare population of granulocytes that comprise ~1% of circulating peripheral leukocytes, and they are structurally and phenotypically related to mast cells. Like mast cells, basophils contain abundant granules with pre-formed inflammatory mediators that can be immediately released upon crosslinking of FcεRI. Important new reagents have recently been used to show that basophils and mast cells have functionally unique and non-overlapping roles in IgE-mediated and IgG-mediated allergic inflammation in mice. In addition, it has been suggested that basophils are central to the differentiation of T helper 2 (T₂) cells. Indeed, a number of studies have suggested that basophil-derived IL-4 is crucial for the development of T₂ cell responses to cytokine proteases, allergens and extracellular parasites.

**What are the key properties of an antigen-presenting cell?**

Burnet’s ‘clonal selection’ theory presupposes that the T cell repertoire contains a vast number of T cell clones expressing unique antigen receptors. Thus, an effective adaptive immune response requires a second cell to select and expand those few T cell clones that express ‘useful’ antigen receptors. Ralph Steinman termed this quality ‘immunogenicity’ (Ref. 145), and crucial work by McDevitt, Sela and Humphrey showed that immunogenicity required ‘immune response’ genes, which mapped to the MHC locus. Zinkernagel and Doherty first demonstrated that the presentation of viral antigens was MHC restricted and multiple laboratories subsequently showed that MHC class I and class II proteins present peptide fragments that are derived from antigens to the T cell receptor (TCR). Thus, immunogenicity requires that proteins are processed and presented on MHC molecules to be recognized by T cells. However, T cell activation requires more than the simple expression of MHC molecules by antigen-presenting cells (APCs). The activation of a naive T cell requires interaction with an APC that provides multiple signals (see the figure): ‘signal 1’ is delivered through interaction of the TCR with peptide–MHC complexes; ‘signal 2’ involves co-stimulatory molecules; and ‘signal 3’ is mediated by instructive cytokines. The ability to deliver these three signals is the defining characteristic of a professional APC.

The search to identify APCs focused on cells that could induce B cell and T cell responses in vitro and in vivo. This antigen-presenting function was initially ascribed to macrophages, because the crucial ‘accessory cell’ found in these assays was adherent, as are macrophages. However, in 1973, Steinman and Cohn identified a different population of adherent cells with large pseudopods in the mouse spleen; they subsequently demonstrated that these ‘dendritic cells’ (DCs) were the most potent population of adherent cells with large pseudopods in the mouse spleen; they subsequently demonstrated that these ‘dendritic cells’ (DCs) were the most potent inducers of T cell proliferation in primary mixed lymphocyte responses. Additionally, DCs are particularly well adapted to process proteins into the peptides that are presented by MHC class II molecules. They express the MHC class II structural α- and β-chains; however, they also express the associated chaperone invariant chains, HLA-DM and HLA-DO, which regulate peptide loading, and the complement of lysosomal proteases and cathepsins that can operate in the acidic phagosomal pathway (recently reviewed in Ref. 154). Although many analyses probe for expression of the invariant chain (II), HLA-DM and HLA-DO, there have been very few stringent evaluations of the phagosomal pathways of atypical APCs.

Basophils as APCs. Although basophil-derived IL-4 can skew T cells to a T₂ cell phenotype, it had been presumed that antigen-specific T cell activation by professional APCs, such as DCs, is still necessary for T₂ cell induction. To examine the requirement for DCs in antigen presentation during T₂ cell differentiation, a number of groups have used mice expressing the human diphtheria toxin receptor (DTR) under the Cd11c promoter (Cd11c–DTR mice); in these mice, diphtheria toxin treatment results in the ablation of CD11c-expressing cells. When bone marrow from Cd11c–DTR mice was used to reconstitute irradiated wild-type mice, diphtheria toxin treatment of these chimeric mice did not alter T₂ cell responses to papain or Trichuris muris. Furthermore, papain injection or T. muris infection of mice in which MHC class II expression was restricted to DCs did not result in a T₂ cell response. Together, these results suggested that MHC class II expression by DCs alone was neither necessary nor sufficient for T₂ cell induction.

This suggested that another MHC class II-expressing cell type might be required for T₂ cell induction in these models. Indeed, basophils were found to constitutively express MHC class II, CD80 and CD86, and the expression of these molecules was further upregulated by activation with IL-3 (Ref. 23) or papain. Moreover, co-culture of ovalbumin (OVA)-specific naive CD4⁺ T cells with antigen-pulsed basophils alone was sufficient to induce T cell proliferation and T₂ cell differentiation in vitro. Basophils could also present antigens to T cells in vivo, as antigen-specific naive CD4⁺ T cells displayed a T₂ cell phenotype in CITTA-deficient mice (which lack MHC class II expression) injected with peptide-pulsed basophils. Basophils were found to effectively incorporate soluble but not particulate antigens by macroinocytosis, which was further enhanced by antigen-specific IgE molecules bound to FcεRI on basophils. IgE-coated basophils efficiently incorporated specific antigens in vivo, and the addition of antigen-specific IgE molecules to CD4⁺ T cell and basophil co-cultures augmented the induction of T₂ cells. However, it is unclear whether the augmented T₂ cell response mediated by IgE was owing to enhanced antigen presentation or increased IL-4.

### Box 1 | What are the key properties of an antigen-presenting cell?

- **MHC class II**: Peptide
- **Peptide**: TCR
- **Signal 1**: Antigen-specific interactions
- **Signal 2**: Co-stimulatory molecules
- **Signal 3**: Instructive cytokines
- **Activated DC**: CD40, CD40 ligand; DAMP, damage-associated molecular pattern; IL-12, interleukin-12; IL-12R, IL-12 receptor; PAM, pathogen-associated molecular pattern; PRR, pattern recognition receptor.
Box 2 | A confounding role for exosomes?

Naïve T cells are usually activated by T cell receptor (TCR)-dependent contact with peptide–MHC complexes on intact antigen-presenting cells (APCs). However, APCs may also release antigen-presenting vesicles, or exosomes. Exosomes are secreted membrane vesicles that form within late multivesicular endosomal compartments and are released into the environment following fusion of the multivesicular bodies with the plasma membrane.

Exosomes can be secreted by numerous cell types including dendritic cells (DCs), B cells, mast cells and microglial cells. They are also secreted by several different tumour cell types. Intestinal epithelial cells (IECs) may also secrete MHC class II-bearing exosomes; putative exosomes carrying IEC-specific proteins have been identified by immunogold analysis of intestinal tissue sections135. Immunologically active exosomes were first described by Thery and Amigorena136,137, who noted that exosomes derived from DCs contain immunologically relevant proteins such as MHC class I, MHC class II and CD86, as well as the heat shock protein HSC73, which is involved in peptide delivery. Exosomes bearing peptide–MHC complexes can stimulate naïve T cells in vitro; however, Amigorena and his colleagues have suggested that exosomes must be recaptured by endogenous DCs for antigen presentation to naïve T cells.

We discuss in the main text how mast cells may contribute to the immune response via the transfer of exosomes to DCs. Similarly, MHC class II-loaded exosomes may be secreted by IECs and act as sources of antigen for tissue-resident or migratory DCs138,139. Thus, the interpretation of many in vivo studies on the immunological functions of different cell types — including some types of DCs — is confounded by the possibility that peptide–MHC complexes from non-conventional cells may simply be transferred to DCs via exosomes for presentation to T cells.

Mixed lymphocyte responses
A tissue-culture technique for testing T cell reactivity. The proliferation of one population of T cells — induced by exposure to inactivated MHC-mismatched stimulator cells — is determined by measuring the incorporation of 3H-thymidine into the DNA of dividing cells.

Macropinocytosis
A type of endocytosis (or phagocytosis) that occurs during the engulfment of apoptotic cells. During macropinocytosis, large droplets of fluid are trapped within the membrane protrusions (ruffles) or phagocytic arms.

production by FcεRI-activated basophils. Together, these results suggested that basophils are a source of IL-4 and may act as the primary APC in the activation of T cells in a variety of T4-Type immune settings.

Different findings in different systems. More recently, the view that basophils might be the primary APC for T2 cell responses in vivo has been challenged by studies suggesting that previous results may have been confounded by the depletion methods that were used. First, the ablation of DCs in Cd11c–DTR mice — as opposed to in wild-type chimeric mice reconstituted with Cd11c–DTR bone marrow — markedly reduced the papain-induced T2 cell response51. As a proportion of skin-resident migratory DCs are radioresistant54–57, diphtheria toxin treatment of wild-type mice reconstituted with bone marrow from Cd11c–DTR mice could have spared migratory DCs in the skin that were sufficient to induce T2 cell responses. Similarly, Cd11c–A3 mice lack MHC class II expression on migratory skin Langerhans cells and dermal DCs, and this may confound the findings from studies using these mice51. Indeed, surgical excision of the injection site a few hours after antigen challenge also attenuated the T2 cell response, suggesting that radioresistant skin DC populations — either Langerhans cells or dermal DCs — might be important51. As Langerhans cell depletion by expressing DTR under the control of the promoter of the gene encoding langerin (also known as CD207) had no effect on T2 cell induction, it was concluded that dermal DCs were responsible for antigen presentation in this setting51. Dermal DCs incorporated the largest amount of injected antigen and were the most potent at inducing T cell proliferation in ex vivo co-culture experiments51. However, DCs alone were unable to skew T cells to a T4,2 cell phenotype ex vivo and required the addition of basophils to the co-cultures51. Thus, it was concluded that although DCs present antigen to T cells, basophils were required for T4,2 cell polarization.

A similar caveat to these models lies in the basophil ablation methods that were used. In a house dust mite (HDM) allergen model, which induces a T4,2 cell response in the lungs, different results were obtained when basophils were depleted using different antibodies — namely, MAR-1 and Ba103141. Treatment with MAR-1, which is specific for FcεRI, had a stronger effect than Ba103 (which recognizes CD200R3) in attenuating T4,2 cell polarization in HDM-challenged mice, although both antibodies reduced basophil numbers to an equivalent extent142. The authors showed that in addition to basophils, MAR-1 also ablated a population of FcεRI+ DCs, whereas Ba103 only depleted basophils. Furthermore, basophils from HDM-challenged mice were poor at presenting antigen ex vivo compared to FcεRI+ DCs isolated from the same lymph nodes, suggesting that DCs mediated antigen presentation. This argument was supported by experiments showing that diphtheria toxin treatment of Cd11c–DTR mice abrogated T4,2 cell responses upon HDM challenge51. Two other groups also questioned the role of basophils in T4,2 cell responses by using novel mouse strains that constitutively lack basophils owing to mast cell protease 8-driven expression of Cre recombinase143,144. Papain-induced T4,2 cell responses were intact in these basophil-deficient mice but not in DC-deficient mice, suggesting that DCs but not basophils had an important role in papain-induced T4,2 cell polarization145,146. These results are in contrast to the aforementioned studies demonstrating an important role for basophils in papain-induced T4,2 cell polarization51,144,145. However, a more recent study targeted DTR expression to basophils under the control of regulatory elements in the gene encoding IL-4 and found that basophils were necessary for T4,2 cell differentiation to peptide antigens, but dispensable for priming to protein antigens147. It is clear that the immunological effects of acute or constitutive deletion of basophils are quite dependent on the method of depletion and the biological setting.

Do human basophils show APC functions? Given the contradictory data generated in mouse systems, researchers soon began exploring the antigen-presenting capabilities of human basophils. In humans, basophils comprise <1% of circulating granulocytes and they express FcεRI, CD203c (also known as ENP33) and CD123 (also known as IL-3Rα). Resting blood basophils are HLA-DR negative and early studies showed that short-term activation with allergens, FcεRI engagement or TLR2 ligands did not induce MHC class II expression51–53. However, subsequent studies suggested that human basophils could express MHC class II in certain disease states (for example, in patients with lupus nephritis), in inflamed tissues that had high levels T4,2-type cytokines54, and in response to culture with IL-3 (REF. 65). However, even these cultured human basophils could not present allergens55 or exogenous peptides to induce the activation of human T cells, perhaps because they
lacked expression of relevant co-stimulatory molecules\textsuperscript{65}. It remains possible that the bone marrow-derived and tissue basophils that are purified from mice are not well represented among the blood-derived human basophils that were used in these studies. Nonetheless, there is not yet any compelling evidence that human basophils can present antigens to CD4\textsuperscript{+} T cells.

**Eosinophils as APCs**

Eosinophils are a population of circulating granulocytes that have long been associated with T\textsubscript{H}2 cell responses. They are elicited in response to IL-5 production during allergic inflammation and parasitic infections\textsuperscript{66–69}, and they are equipped with an arsenal of cationic proteins and inflammatory mediators with antihelminth effector functions\textsuperscript{70}. In addition to their effector role, eosinophils may also be involved in the modulation of T\textsubscript{H}2 cell immune responses. Similar to basophils, eosinophils have constitutive activity at the IL-4 locus\textsuperscript{38–40,71}, and they are the major source of IL-4 upon challenge with *Schistosoma mansoni* eggs\textsuperscript{72}. Moreover, eosinophils release chemokines that are crucial for the recruitment of T cells to the lungs during allergic airway hyperresponsiveness\textsuperscript{73–75}.

Like mast cells and basophils, eosinophils can also express MHC class II. Expression of MHC class II by eosinophils was first reported in sputum and bronchoalveolar lavage samples from patients with asthma in the early 1990s\textsuperscript{75,76}. Although freshly isolated eosinophils from blood were devoid of MHC class II expression, its expression could be induced following *in vitro* culture with activated T cell-conditioned media, GM-CSF, IL-3, or a combination of IL-3 and IFN\gamma\textsuperscript{77–80}. Similar observations have been made in mice, where airway or lymph node eosinophils constitutively express MHC class II\textsuperscript{81–84}. Although mouse eosinophils in the peritoneal cavity are MHC class II negative, expression of MHC class II is induced following the culture of these cells with GM-CSF\textsuperscript{85–87}. HLA-DR in human eosinophils localizes to detergent-resistant lipid rafts that have been suggested to enhance antigen presentation by professional APCs\textsuperscript{88} and, in both humans and mice, MHC class II-expressing eosinophils are capable of presenting superantigens, peptides and protein antigens to T cell hybridomas and T cell lines\textsuperscript{79,82,83,86–89}. These data suggest that eosinophils have the potential to function as APCs.

Evidence supporting a role of eosinophils in antigen presentation *in vivo* came from studies demonstrating that eosinophils elicited by repeated airway antigen challenge migrate from endobronchial areas to the draining mediastinal lymph nodes\textsuperscript{81}. These eosinophils were predominantly found in the T cell zones of the draining lymph nodes and they formed clusters with antigen-specific T cells\textsuperscript{82}. Lymph node eosinophils expressed MHC class II, CD80 and CD86 and could restimulate memory T cells from antigen-challenged mice\textsuperscript{81,89}. Moreover, intratracheal transfer of antigen-pulsed eosinophils resulted in the enhanced proliferation of antigen-experienced CD4\textsuperscript{+} T cells in the draining lymph nodes, suggesting that eosinophils could induce antigen-specific T cell proliferation. Similar results were obtained using mice adoptively transferred with antigen-specific naive T cell receptor (TCR)-transgenic T cells, suggesting that eosinophils could also activate naive T cells\textsuperscript{87}. The activation of T cells by eosinophils seemed to be MHC class II dependent, as the intraperitoneal injection of *Strongyloides stercoralis* antigen-loaded wild-type eosinophils, but not MHC class II-deficient eosinophils, resulted in the increased production of T\textsubscript{H}2 cell-associated cytokines\textsuperscript{89}.

However, some studies have argued that eosinophils might be incapable of efficiently processing protein antigens. Although human eosinophils expressed MHC class II after IL-3 stimulation *in vitro* and could stimulate antigen-specific primed T cells when pulsed with peptides, they were unable to do so when pulsed with whole antigen\textsuperscript{89}. Similarly, whole-protein-pulsed mouse eosinophils could not stimulate antigen-specific naive T cells, although some proliferation of T cells was observed when the eosinophils were pulsed with peptides\textsuperscript{84}. A recent study contradicted these findings and showed that mouse eosinophils were in fact efficient APCs for naive antigen-specific T cells both *in vitro* and *in vivo*\textsuperscript{90}. They attributed the previously reported lack of antigen-processing ability of eosinophils to the use of NH\textsubscript{4}Cl to lyse red blood cells. In their experiments, eosinophils were unable to activate antigen-specific T cells if they were exposed to NH\textsubscript{4}Cl, potentially owing to its lysosomotropic activity. Although this method was used by one of the previous studies\textsuperscript{89}, red blood cells were removed by density gradients and centrifugation in the other\textsuperscript{80}. Alternatively, differences in the human and mouse systems may contribute to the discrepancies that were seen in the latter study.

Although some controversy still remains as to their antigen-processing ability, many studies have convincingly demonstrated that MHC class II and co-stimulatory molecules are expressed by airway-associated and lymph node eosinophils, and by cytokine-activated circulating eosinophils\textsuperscript{97–98}. Moreover, eosinophils localize to the same areas as classical APCs, as they migrate to T cell zones in the lymph nodes during allergic airway hyperresponsiveness reactions\textsuperscript{91,97}. Recently, mice that express DTR driven by eosinophilic peroxidase have been generated, but T cell-dependent responses in such mice have only been analysed in an asthma model. At least in an allergic airway disease model, eosinophil depletion had no effect during the antigen sensitization phase\textsuperscript{92}, suggesting that T cell responses are intact in the absence of eosinophils. The generation of new methods to selectively ablate MHC class II expression on eosinophils will be helpful to further investigate the *in vivo* role of eosinophils as APCs in other disease settings.

**APC functions of neutrophils**

So far, we have examined the potential antigen-presenting functions of mast cells, basophils and eosinophils. However, neutrophils are the most abundant type of granulocyte and are worth considering. Neutrophils are rapidly recruited to sites of tissue damage where they extrude neutrophil extracellular traps (NETs), produce antimicrobial peptides, phagocytose microorganisms
and recruit other immune cells to clear pathogens. Thus, neutrophils are generally regarded as professional phagocytes that are involved early in the response to tissue injury and infection. However, there is increasing evidence that neutrophils may also modulate the adaptive immune response through the production of chemokines and cytokines that recruit DCs to sites of inflammation\(^1\). Thioglycollate-elicited peritoneal neutrophils in mice express CD80, but neither CD86 nor MHC class II\(^2\). However, co-culture with CD4\(^+\) T cells may lead to a minimal level of cell-surface MHC class II expression and the ability to process OVA protein and activate OVA-specific CD4\(^+\) T cells\(^3\). Similarly, neutrophils purified from the colons of colitic mice were MHC class II\(^+\) and, when pulsed with OVA peptide, could also activate CD4\(^+\) T cells. Human neutrophils could express HLA-DR and could stimulate superantigen-dependent T cell activation; however, they could not re-activate tetanus toxoid-specific T cells\(^4\).

Despite the suggestion that neutrophils may be professional APCs, the interpretation of these data is quite complicated. First, although neutrophil preparations in these studies are 95–98% pure, the presence of only a few contaminating DCs in either the neutrophil or T cell preparation could be sufficient to mediate DC-driven T cell activation. Second, neutrophils induced by *Pseudomonas aeruginosa* airway infection lack MHC class II expression, despite expressing CD80 and CD86 (REF. 96). Finally, it is notable that in many of these reports, MHC class II expression occurs in GM-CSF-rich environments. In this setting, these ‘differentiated’ neutrophils could easily be confused with inflammatory TNF and iNOS producing (TIP)-DCs\(^7\) or a recently described ‘neutrophil–DC hybrid’ cell that shows characteristics of both cell types\(^8\). Thus, there is not compelling evidence that neutrophils can function as true APCs.

**ILCs and CD4\(^+\) T cells as APCs**

In recent years, there has been growing interest in ILCs, which function as rapid sources of cytokines early during immune responses. ILCs lack rearranged antigen receptors but otherwise resemble CD4\(^+\) T cells in their developmental pathways, transcription factor profiles and cytokine-producing patterns. Type 1 ILCs (ILC1s), ILC2s and ILC3s produce T\(_{h1}\), T\(_{h2}\) and T\(_{h17}\)-type cytokines, respectively. Recent evidence implicates ILCs as crucial regulators of innate immunity and inflammation at barrier surfaces, including the skin, airways and gastrointestinal tract (reviewed in REF. 99).

In addition to their early effects on innate immune responses, there is increasing evidence that ILCs may directly interact with CD4\(^+\) T cells. One group reported that ILC3s can express CXC-chemokine receptor 5 (CXCR5) and CC-chemokine receptor 7 (CCR7), and localize to secondary lymphoid organs where they may control the maintenance of memory CD4\(^+\) T cells\(^10\). More directly, genome-wide transcriptional profiling of ILC3s showed that they express genes that encode MHC class II structural components, as well as proteins that are necessary for antigen presentation, including li and H2-DM\(^12\). In this report, a subset of ILC3s had surface expression of MHC class II and could acquire, process and present exogenous antigens in *vitro* but did not induce proliferation of CD4\(^+\) T cells. It was suggested that ILCs probably do not activate naive CD4\(^+\) T cells; nevertheless, cell-specific deletion of MHC class II expression in retinoic acid receptor-related orphan receptor-yt (RORyt)-expressing ILC3s led to the dysregulation of CD4\(^+\) T cells specific for commensal bacteria and the loss of intestinal epithelial integrity\(^10\). Thus, these authors proposed that MHC class II expression on ILCs contributed to intestinal homeostasis by countering professional APCs to prevent the inappropriate activation of commensal-specific CD4\(^+\) T cells. In contrast with these initial observations, a second laboratory examined the function of splenic ILC3s from the same strain of mice lacking expression of MHC class II in ILC3s\(^10\). Interestingly, in a different animal facility, this strain of mice did not develop any intestinal pathology, suggesting a crucial role for microbial exposure\(^10\). They also reported that, in contrast to ‘tolerizing’ intestinal ILC3s, splenic ILC3s respond to IL-1\(p\) by upregulating both MHC class II and co-stimulatory molecules, and thus contribute to CD4\(^+\) T cell proliferation. Perhaps ILC3s localized to different environments have different effects on CD4\(^+\) T cell activation and differentiation.

In agreement with these observations, we recently showed that CD4\(^+\) T cells regulate the number and function of ILC3s in the mouse intestinal lamina propria in an MHC class II-dependent manner\(^10\). Although we did not show that this was directly regulated by MHC class II expression on the ILCs, we did find that the level of MHC class II expressed by ILC3s in the intestinal lamina propria was determined by the presence of functional CD4\(^+\) T cells\(^10\). Thus, MHC class II-expressing ILC3s may regulate and also be regulated by CD4\(^+\) T cells.

There may also be such cross-regulation between ILC2s and T\(_{h2}\) cells. ILC2s were first characterized as cells that expressed MHC class II and inducible T cell co-stimulator (ICOS)\(^10\). They are resident in the lungs and other mucosal tissues, and IL-2 produced by T\(_{h2}\) cells may enhance cytokine production by ILC2s\(^10\). More importantly, purified lung ILC2s were shown to present peptide to TCR-transgenic CD4\(^+\) T cells in *vitro*\(^10\); however, ILC2s could not present OVA protein in this study. A more recent examination found that peptide-loaded MHC class II\(^+\) ILC2s induced T\(_{h2}\) cell differentiation in *vitro*; ILC2s could also internalize and process protein antigens, but *in vitro* CD4\(^+\) T cell proliferation could not be detected\(^10\). Nonetheless, MHC class II\(^+\) ILCs contributed to the clearance of intestinal helminths\(^10\). The study of MHC class II-dependent functions of ILC2s and ILC3s is a rapidly evolving area and the data remain contradictory. Importantly, the systems studied to date involve ablation of cell-specific MHC class II expression rather than directly assaying the APC function of ILC3s in the absence of antigen presentation by DCs.

Among other non-B cell lymphocytes, CD4\(^+\) T cells also have the ability to express MHC class II and present antigens to other CD4\(^+\) T cells. Although CD4\(^+\) T cells from mice do not express MHC class II, human CD4\(^+\) T cells express HLA-DR molecules upon TCR
activation\textsuperscript{108,109}. Antigen presentation by CD4\textsuperscript{+} T cells seems to have a dominant tolerance-inducing effect on other CD4\textsuperscript{+} T cells. Peptide-loaded T cell clones can activate each other to induce proliferation. However, these T cells are defective in response to restimulation\textsuperscript{110}, suggesting that antigen-presenting CD4\textsuperscript{+} T cells induce anergy. As MHC class II molecules on CD4\textsuperscript{+} T cells are loaded with self peptides\textsuperscript{111}, it has been postulated that activation by HLA-DR molecules expressed by CD4\textsuperscript{+} T cells may serve to limit self peptide-reactive CD4\textsuperscript{+} T cells. The lack of MHC class II expression by mouse CD4\textsuperscript{+} T cells has hampered progress in investigating the antigen-presenting role of CD4\textsuperscript{+} T cells.

**Non-haematopoietic cells as APCs**

Up to this point, we have focused on haematopoietic cells — predominantly myeloid cells — that might act as APCs. However, there is an extensive literature on the antigen-presenting abilities of radioresistant endothelial cells and epithelial cells in particular clinical settings, especially autoimmunity and transplant tolerance (FIG. 2).

**Lymph node stromal cells.** The T cell–APC interactions that mediate T cell activation do not occur in free space but in tightly regulated anatomic regions of lymph nodes, which are defined by the presence of non-haematopoietic stromal cells of mesenchymal and endothelial origin. LNSCs can be divided into subclasses on the basis of their surface expression of the glycoproteins CD31 (also known as PECAM1) and podoplanin (also known as GP38), and their localization within lymph nodes. These subclasses include fibroblastic reticular cells (FRCs), follicular DCs (FDCs), lymphatic endothelial cells (LECs), blood endothelial cells (BECs), pericytes and a small proportion (<5%) of otherwise undefined stromal cells. For many years, it was presumed that the sole function on non-FDC LNSCs was to provide the structural ‘backbone’ for secondary lymphoid organs. However, the stroma clearly contributes to adaptive responses (as reviewed in REF. 112) by concentrating antigens in the lymph nodes and directing DC trafficking. Additionally, multiple groups have demonstrated that LNSCs ectopically express tissue-specific antigens, including transgene-directed neoantigens and endogenous tyrosinase, and that they can induce the deletion of self-reactive CD8\textsuperscript{+} T cells\textsuperscript{113–117}. The mechanisms for the expression of tissue-specific antigens are not completely clear. Thymic medullary epithelial cells express autoimmune regulator (AIRE), which is a transcriptional activator that promotes the expression of tissue-specific antigens to regulate central deletional tolerance. The Anderson laboratory used an Aire-driven transgenic reporter to identify a population of lymph node cells that expressed AIRE and tissue-specific antigens\textsuperscript{118}. Interestingly, these AIRE\textsuperscript{+} cells expressed low levels of CD45 and CD11c, and were radiosensitive, suggesting that they were a haematopoietic population that was distinct from other LNSCs\textsuperscript{117}. It is not clear if AIRE is expressed at significant levels in LNSCs, although a related transcriptional regulator, deformed epidermal autoregulatory factor 1 (DEAF1), may be\textsuperscript{114,115}.

LNSCs could either be intrinsically tolerogenic cells or may simply express MHC class I in the absence of co-stimulatory molecules and, as such, induce the deletion of self-reactive CD8\textsuperscript{+} T cells\textsuperscript{113,114}. However, the ImmGen consortium has shown that FRGs, LECs and BECs can upregulate surface expression of MHC class II molecules under inflammatory or infectious conditions\textsuperscript{114}. In this setting, it has been demonstrated that peripheral AIRE\textsuperscript{+} cells, but not radioresistant LNSCs, can also mediate deletional tolerance of autoreactive CD4\textsuperscript{+} T cells\textsuperscript{117}. Importantly, there are no data suggesting that CD4\textsuperscript{+} T cell deletion induced by either LNSCs or AIRE\textsuperscript{+} cells is mediated independently of DCs. Indeed, a recent report suggested that LNSC-mediated deletion of CD4\textsuperscript{+} T cells is dependent on peptide–MHC class II complexes that are acquired from DCs\textsuperscript{119}. Thus, it is possible that these cells are similar to basophils in modulating antigen presentation by DCs.
**Endothelial cells and epithelial cells.** Both human and mouse vascular endothelial cells and tissue-resident epithelial cells can express MHC class I and class II molecules, and there is some evidence that interactions with CD4+ T cells may be involved in some autoimmune diseases and in graft rejection. Vascular endothelial cells in human allografts can express both MHC class I and class II molecules, and can present processed protein antigens to T cell clones. However, there is no evidence either in vitro or in vivo that human endothelial cells can express CD80 or CD86, which are necessary to stimulate naive alloreactive T cells. Both rat and mouse allograft models suggest that DCs in the graft (‘passenger leukocytes’) are necessary to initiate graft rejection. However, memory cells have decreased requirements for co-stimulation and one group has shown in xenograft models that human memory T cells — specifically the effector memory subset — can directly respond to graft vascular endothelium to mediate rejection.

Epithelial cells — including intestinal epithelial cells (IECs), airway epithelial cells and keratinocytes — are uniquely positioned at the interface between the host immune system and an environment teeming with antigens, including pathogenic microorganisms and food antigens, as well as commensal microorganisms. Thus, decisions about whether to generate pro-inflammatory or tolerizing responses must continuously be made at mucosal surfaces. Similar to vascular endothelial cells, epithelial cells can express MHC class II and may be uniquely poised to regulate T cell responses to mucosal antigens. Most work has focused on IECs and lung alveolar epithelial cells; however, we have previously suggested that MHC class II expression restricted to keratinocytes could mediate autoimmune skin disease.

Multiple reports suggest that both mouse and human ileal IECs constitutively express MHC class II molecules. Do these cells have the machinery to present antigen? Much of the work suggesting that IECs can process and present antigen has been limited to in vitro studies using cell lines or primary cells treated with IFNγ. These studies do show that in the presence of IFNγ, the appropriate machinery — including Ii, H2-DM and proteases — can be expressed by IECs in the small intestine and by oesophageal epithelial cells. Interestingly, IECs isolated from patients with inflammatory bowel disease (IBD) or from animal models of IBD express higher levels of MHC class II. Work using IEC-like cell lines has shown that these cells can stimulate T cell hybridomas or clones in vitro. However, the evidence that naive T cells can be stimulated by IECs in vivo is less compelling. Indeed, there is no clear evidence that IECs express sufficient levels of the appropriate co-stimulatory molecules to activate naive CD4+ T cells.

There are accumulating data suggesting that IECs may contribute to the differentiation of Treg cells and the maintenance of tolerance. Neoantigen targeted to IECs induced the expansion of antigen-specific Treg cell populations in a manner that was independent of DC depletion. A more recent in vivo study examining transgenic mice in which MHC class II expression was restricted to either IECs or DCs showed that antigen presentation by DCs, but not by IECs, could drive CD4+ T cell-dependent intestinal inflammation. The requirement for MHC class II was examined by selectively deleting expression of CIITA in non-haematopoietic cells, including IECs. In these mice, colitis induced by IL-10 blockade was much more severe than in co-housed wild-type littermates; disease was attributed to increased numbers of local Treg cells and an imbalance in the relative numbers of effector T cells and Treg cells. Similar to investigations of IECs in the small intestine (the focus of most published work), it was also found that MHC class II colonic epithelial cells could not stimulate naive CD4+ T cells in vitro; rather, they suppressed the activation of CD4+ T cells by professional APCs. The mechanism for this was not clear, although it was independent of transforming growth factor-β.

In both mice and humans, as many as half of the MHC class II+ cells in the lungs are of non-haematopoietic origin. Non-haematopoietic cells include vascular and alveolar epithelial cells. Most data suggest that adaptive immunity to respiratory pathogens is predominantly mediated by haematopoietic APCs, including migratory DCs and alveolar macrophages; however, there are data suggesting that epithelial cells might modulate these responses. For example, type II alveolar epithelial cells comprise only 4% of the epithelium but they are strategically located to respond to airborne pathogens, and they produce antimicrobial peptides and pro-inflammatory mediators such as complement components. Type II alveolar epithelial cells in both mice and humans express MHC class II that can be upregulated by IFNγ and microbial stimulation. Both endogenous neo-self antigens and exogenous antigens — for example, from Bacille Calmette–Guérin (BCG) — can be processed and presented by type II alveolar epithelial cells. MHC class II expression on pulmonary non-haematopoietic cells may contribute to decreased inflammation and acceptance of orthotopic lung transplants. In transgenic systems, type II alveolar epithelial cells can prime antigen-specific CD4+ T cells. Interestingly, the T cell response is skewed towards differentiation of forkhead box P3 (FOXP3)-expressing CD4+ T cells. In this regard, type II alveolar epithelial cells may be similar to vascular endothelial cells. Thus, endothelial cells and epithelial cells may be additional cell types that express MHC class II and modulate the outcome of DC–CD4+ T cell interactions.

**Concluding remarks.** There are a large number of haematopoietic and non-haematopoietic cell types that can express MHC class II molecules and present antigens to CD4+ T cells. However, MHC class II expression alone is not sufficient for full APC function, as APCs need to be able to process antigens, migrate to secondary lymphoid organs and express co-stimulatory molecules. In this regard, professional APCs, such as macrophages and B cells, could potentially replace the function of DCs in certain situations. However, the non-professional APCs that have been discussed in this Review are unlikely to replace DCs, because there is little compelling data (if any) that these cell types are able to activate naive CD4+ T cells.
Rather, we propose that these non-conventional APCs modulate immune responses that have been initiated by DCs. This is especially true for non-haematopoietic cells that may mediate the deletion of autoreactive T cells or may stimulate T<sub>reg</sub> cells. The requirement for additional non-DC APCs is quite context-dependent — for example, basophils, mast cells and eosinophils have been implicated in the induction of T<sub>2</sub> cell responses, whereas CD4<sup>+</sup> T cells, and stromal, endothelial and epithelial cells may contribute to tolerance. Studies in mice clearly require careful analyses of the cell types that are affected by manipulation. Similarly, increasing study of human tissues other than blood will be necessary to validate findings from mouse systems.

22. This study shows that MHC class II expression on mast cells is induced by TLR agonists and IFNγ, and can support the activation of effector T cells and T<sub>reg</sub> cells but not that of naïve T cells.
26. References S, 4, and 23 show that basophils express MHC class II and present antigens to T cells to promote T<sub>2</sub>‑type responses.
28. This study argues that FcεRI‑expressing DCs and not basophils are responsible for antigen presentation to Th type 2 response.
30. Gong, J. et al. The antigen presentation function of bone marrow‑derived mast cells is spatiotemporally restricted to a subset expressing high levels of cell surface FcεRI and MHC II. *BMC Immunol. 11, 34 (2010)*.
35. Tkaczyk, C. et al. Specific antigen targeting to surface IgE and IgG on mouse bone marrow‑derived mast cells enhances efficiency of antigen presentation. *Immunology 94, 318–324 (1998)*.
38. This study argues that basophils are important for papain‑induced T<sub>2</sub> cell responses not for their APC function but for their effects on DCs.


64. References 61 and 62 were the first studies to propose that basophils might not act as APCs in the setting of human allergy. Sharma, M. et al. Circulating human basophils lack the features of professional antigen presenting cells. Sci. Rep. 3, 1188 (2013).


74. This study suggests that some APCs termed LNSCs are actually bone marrow-derived population of cells that induce T cell anergy. Malthotra, D. et al. Transcriptional profiling of stroma from flamed and restituted Ddx4-/- testes identifies immunological hallmarks. Nature Immunol. 13, 499–510 (2012).

75. This preliminary analysis dissects which LNSCs can and do express MHC class II that is inducible by inflammatory signals. Dubrot, J. et al. Lymph node stromal cells acquire peptide-MHCII complex expression in response to chronic inflammatory signals. J. Exp. Med. 211, 1153–1166 (2014).

76. This study suggests that high levels of expression endogenous MHC class II molecules and can also acquire peptide–MHC–II complexes from DCs. Hughes, C. et al. Lymphoid endothelial cells express CD2 and CD45. J. Exp. Med. 171, 1455–1467 (1995).

REVIEWS


References 123 and 124 are two of many articles from the Pober laboratory examining the function of MHC class II+ endothelial cells as APCs during graft acceptance or rejection. Many of the articles from this laboratory examine the mechanisms for stimulating memory but not naive CD4+ T cells.


References 130, 132 and 133 are among the first manuscripts to demonstrate that IECs express MHC class II and present protein antigen to CD4+ T cell hybridomas.


This study suggests that IECs may generate immunologically active exosomes.


This is one of the first articles demonstrating that exosomes may contribute to CD4+ T cell activation.


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Competing interests statement

The authors declare no competing interests.

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