# Epithelial MHC Class II Expression and Its Role in Antigen Presentation in the Gastrointestinal and Respiratory Tracts

Jonathan E. Wosen[†], Dhriti Mukhopadhyay[†], Claudia Macaubas and Elizabeth D. Mellins*

Program in Immunology, Department of Pediatrics, Stanford University, Stanford, CA, United States

As the primary barrier between an organism and its environment, epithelial cells are well-positioned to regulate tolerance while preserving immunity against pathogens. Class II major histocompatibility complex molecules (MHC class II) are highly expressed on the surface of epithelial cells (ECs) in both the lung and intestine, although the functional consequences of this expression are not fully understood. Here, we summarize current information regarding the interactions that regulate the expression of EC MHC class II in health and disease. We then evaluate the potential role of EC as non-professional antigen presenting cells. Finally, we explore future areas of study and the potential contribution of epithelial surfaces to gut-lung crosstalk.

Keywords: epithelial cells, MHC class II, antigen presentation, intestine, respiratory

## INTRODUCTION

The epithelium serves as both a physical and chemical barrier as well as an absorptive surface. Our understanding of the function of the aerodigestive epithelium has gradually evolved from its role as a static barrier to a dynamic structure regulating multiple processes. In fact, ECs may have primary immune functions that affect the balance between tolerance and inflammation, as evidenced by expression of MHC class II on the EC surface, an area of exploration several decades ago. Recent evidence suggests there may also be communication with the lung from the gut directed by its microbiome. Because host-microbial interactions first occur at the epithelial surface, re-visiting the role of ECs in antigen processing and presentation is timely. This review aims to synthesize current findings on MHC class II expression in the gut and the lung, explore the role of ECs as non-professional antigen presenting cells (APCs) and discuss how this area may be further investigated as a target for potential diagnostic or therapeutic interventions.

### Structure and Function of the Aerodigestive Epithelium

Though the digestive and respiratory systems are thought of as two entirely distinct anatomic cavities, they have a shared developmental origin from the primitive gut (1, 2). The digestive tube initially extends through the length of the body from which the respiratory tube outpouches, sharing a common embryonic chamber called the pharynx. The linings of these primitive tubes are comprised of embryonic endoderm. The digestive tube eventually differentiates into the components of the gastrointestinal tract including esophagus, stomach, small intestine and colon, due to the interaction of endodermal epithelium with regionally specific mesodermal mesenchyme. The respiratory tube bifurcates into two lungs, with laryngotracheal endoderm becoming the epithelial lining of the trachea, bronchi and lung parenchyma, similarly directed by the regional mesenchyme (3).

By birth, the terminal portion of the digestive tract has matured into the small intestine and large intestine. The small intestine is ~5 m in length and composed of three separate segments—duodenum, jejunum, and ileum—that are exposed to dietary antigens and crucial for oral tolerance. Small intestinal epithelium is composed of finger-like projections called villi and invaginations called crypts of Lieberkühn. The large intestine, or colon, comprises the distal 1.5 m of the gastrointestinal tract and histologically lacks villi (4).

The intestinal mucosa is composed of simple columnar epithelium that comprises a surface area of 200–300 m$^2$ (1). This immense structure facilitates the absorption of nutrients in the small intestine and water in the large intestine, while also acting as a barrier and modulator of immunity (5). Intestinal epithelial cells (IECs) include multiple different specialized cells including enterocytes, goblet cells, Paneth cells, enteroendocrine cells, M cells and tuft cells, all of which have discrete functions (**Figure 1**). Enterocytes are the most abundant cell type in the gut epithelium and function to transcytose antimicrobial proteins and IgA as well as absorb nutrients (6). Goblet cells secrete mucus, resistin-like molecule β, which modifies T cell-mediated immunity, and trefoil factor, which promotes epithelial healing after injury; these cells have also been shown to participate in antigen delivery to dendritic cells (DCs) of the submucosa through specialized antigen passages (7, 8). Paneth cells in the small intestine secrete microbicidal proteins including α-defensins, C-type lectins, lysozyme and phospholipase A$_2$; they also sustain

stem cells in the crypts of Lieberkühn to promote epithelial regeneration (6). Enteroendocrine cells secrete neurohormones including gastric inhibitory peptide, glucagon-like peptide, and vasoactive intestinal peptide in response to nutrients in order to regulate motility and digestion (6). M cells (microfold cells), a specialized EC subset derived from enterocytes, transcytose antigens to the underlying gut-associated lymphoid tissue (GALT), the complement of lymphocytes that is composed of intraepithelial and lamina propria lymphocytes (IELs, LPLs) (9–11). Because of their role in antigen uptake, there has been interest in whether M cells present antigens using the MHC class II pathway to facilitate adaptive immunity, though existing evidence is conflicting (12–16). Tuft cells, a rare EC with a distinctive tufted morphology, express chemosensory receptors and may have roles in type 2 immunity and mucosal immunity, but remain poorly understood (17, 18).

IECs have a known role in innate immune responses via expression of a variety of pattern recognition receptors (PRRs). PRRs trigger intracellular pathways that lead to cytokine and chemokine release. PRRs important in the gut include Toll-like receptors 1–9 (TLRs) and nucleotide-binding oligomerization domain-containing proteins (NODs) that recognize pathogen-associated molecular patterns (PAMPs) derived from microbial components (19–21). Interestingly, apical TLRs on the luminal side of the gut (TLR 1 and 2) appear to be hyporesponsive to PAMPs *in vitro*, while the other PRRs expressed in IECs are either endosomal (TLR3–4, 7–9), cytoplasmic (NOD1 and NOD2) or
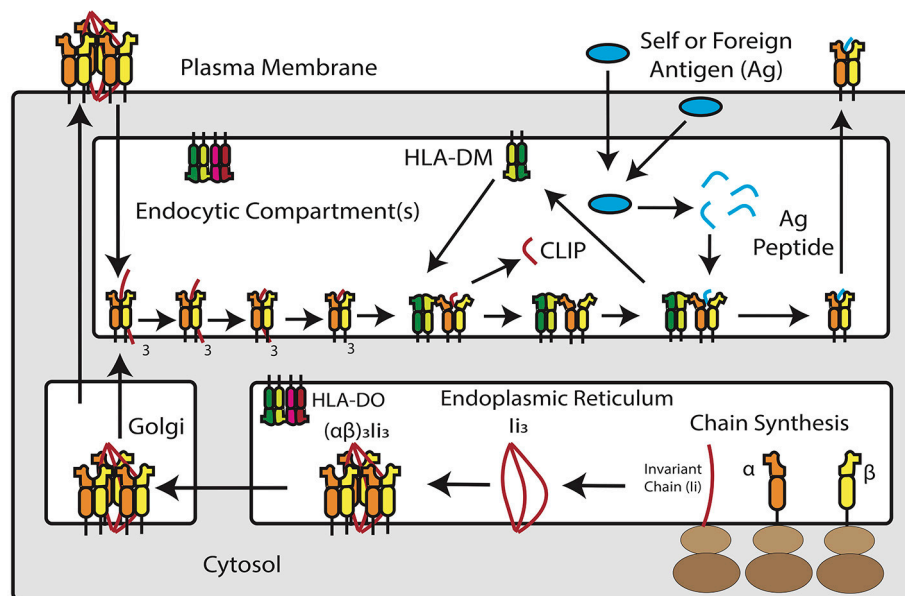


**FIGURE 1 |** Overview of MHC Class II Antigen Presentation Pathway. Newly synthesized MHC class II α and β chains assemble into heterodimers in the endoplasmic reticulum, where they are bound by trimers of invariant chain. MHC class II and invariant chain form nonamers–or, according to recent studies, pentamers and heptamers–that traffic into an acidic endosomal compartment. Within this compartment, invariant chain is degraded down to class II invariant chain-associated peptide (CLIP), which occupies the peptide binding groove of the MHC class II molecule. HLA-DM, a non-classical MHC protein, catalyzes the removal of CLIP in exchange for high-affinity peptide binders derived from extracellular or cytosolic antigens. In a subset of antigen presenting cells, HLA-DM is blocked by HLA-DO, which competitively binds to HLA-DM and prevents it from interacting with MHC class II. Once loaded with peptide, MHC class II molecules traffic to the plasma membrane for inspection by CD4+ T cells.

only on the submucosal basolateral membrane (TLR4 and 5), supporting a role in tolerance to the gut microbiome (21–23).

The pseudostratified epithelium of the airway is ∼100 $m^2$ in surface area and composed of a variety of airway epithelial cells (AECs), many of which are specialized to the lung but homologous to the gut. The bronchial epithelium is composed of basal cells, columnar ciliated epithelial cells, mucous goblet cells, brush or tuft cells and Clara cells (24, 25). The alveolar epithelium, meanwhile, is composed mostly of Type I and Type II pneumocytes (24, 26). Neuroendocrine cells, similar to the gut, promote the vasomotor function of the airways (27). Basal cells, similar to Paneth cells, are important for epithelial regeneration and produce bioactive molecules including endopeptidase, 15-lipoxygenase products and cytokines (28, 29). Goblet cells, like those of the gut, secrete mucus in order to trap foreign particles and pathogens (24, 30). Columnar ciliated cells account for the majority of AECs in the bronchial lumen and are responsible mainly for mucus clearance (31). Clara cells produce surfactants and antiproteases including secretory leukocyte protease inhibitor and p450 mono-oxygenases (32). Type I pneumocytes are mainly responsible for gas exchange and make up the majority of the alveolar surface, though recent evidence suggests they may have additional roles in remodeling, regulation and defense (33). Type II pneumocytes are responsible for surfactant production and reuptake though they also act as progenitor cells and enhance immune responses (33, 34). Unlike the gut, an integrated mucosal immune system does not exist in the healthy adult human lung, though bronchus-associated lymphoid tissue (BALT) is present in young children and also develops in many disease states in adulthood (35, 36). AECs express a similar variety of PRRs as IECs including all known human TLRs, RIG-1-like receptors, NOD-like receptors, C-type lectins and surfactant proteins (26, 37–41).

## Overview of MHC Class II and Costimulatory Molecules

MHC class II molecules are transmembrane αβ heterodimers. In humans, there are three MHC class II isotypes: HLA-DR, HLA-DP, and HLA-DQ, encoded by α and β chain genes within the Human Leukocyte Antigen (HLA) locus on chromosome 6. The expression of MHC class II antigen presentation machinery is tightly regulated by class II transactivator (CIITA), which recruits DNA-binding factors, chromatin modifying proteins, and transcription initiators to the MHC II locus. The class II pathway for processing and presenting antigen is complex but involves interaction with accessory molecules and trafficking through intracellular compartments (42–44). In the ER, nascent MHC class II molecules associate with invariant chain (CD74), a dedicated chaperone protein that directs MHC class II into a low-pH, late-stage endosomal compartment, known as the MHC II compartment (MIIC). Within MIIC, proteases cleave invariant chain and leave a nested set of invariant chain fragments known as class II invariant chain-associated peptides (CLIP) (44). CLIP temporarily occupies the peptide binding groove of the MHC class II molecule. A catalytic protein, HLA-DM, exchanges CLIP

for peptides that bind MHC class II with high-affinity (45). Some APCs, including B cells, thymic epithelial cells and certain DCs, express a regulator of HLA-DM, known as HLA-DO, which competitively inhibits DM-MHC class II interaction (46). Once formed, peptide/MHC class II complexes traffic to the cell surface for interaction with $CD4^+$ T cells through the T-cell receptor, as the first signal to the lymphocyte required to elicit an antigen-specific adaptive response. The MHC class II pathway is shown schematically in **Figure 1**.

Efficient activation of naïve $CD4^+$ T cells requires a second signal to the lymphocyte in the form of co-stimulation to complete the APC-T cell interaction. Classical costimulatory signals include CD80 and CD86, members of the B7 family that interact with stimulatory CD28 or inhibitory CTLA-4 on T cells (47). These molecules are upregulated on professional APCs in response to PAMPs or damage associated molecular patterns (DAMPs), such as ATP (48). T cell recognition of peptide/MHC without sufficient co-stimulation induces a hyporesponsive, anergic state (49).

## POTENTIAL ROLE OF ECS IN ANTIGEN PRESENTATION

### MHC Class II in Health and Disease in Humans

#### Intestine

IECs have been described as capable of MHC class II expression for several decades (50–54). MHC class II, HLA-DM and invariant chain have been reproducibly detected in IECs throughout all segments of the small intestine (12, 52, 54–57). In humans, cell surface expression of class II is first detected around 18 weeks' gestation and increases through development (15, 58, 59). At homeostasis, MHC class II appears to be constitutively expressed on small intestinal enterocytes, most densely in the upper villus (15, 53, 56). Conversely, MHC class II is absent from small intestinal crypts as well as colonic epithelium under normal physiologic conditions but is upregulated in specimens obtained from patients with active inflammatory bowel disease (IBD), celiac disease, and graft vs. host disease (**Figure 2**) (55, 60–66). Exposure to inflammatory antigens, such as gliadin in celiac disease, has also been shown to cause the upregulation of cell surface MHC class II (62, 67). These changes are dependent on active disease; celiac patients in remission have IEC MHC class II levels comparable to those of non-celiac controls (68). IFNγ appears to be the key disease-elevated cytokine that regulates this process (69). In IBD, for example, increased surface MHC class II expression is correlated with increased tissue IFNγ levels (**Figure 2**) (70).

IECs are polarized, with brush border enzymes localized to the apical (luminal) surface to break down dietary antigens and poly-Ig receptors restricted to the basolateral surface to translocate IgA into the intestinal lumen (71). This polarity is important as peptide-presentation to the resident immune cells of the GALT is necessary for systemic crosstalk. Some early tissue staining studies in humans showed predominantly apical expression of MHC class II in IECs (53, 55, 72). However,
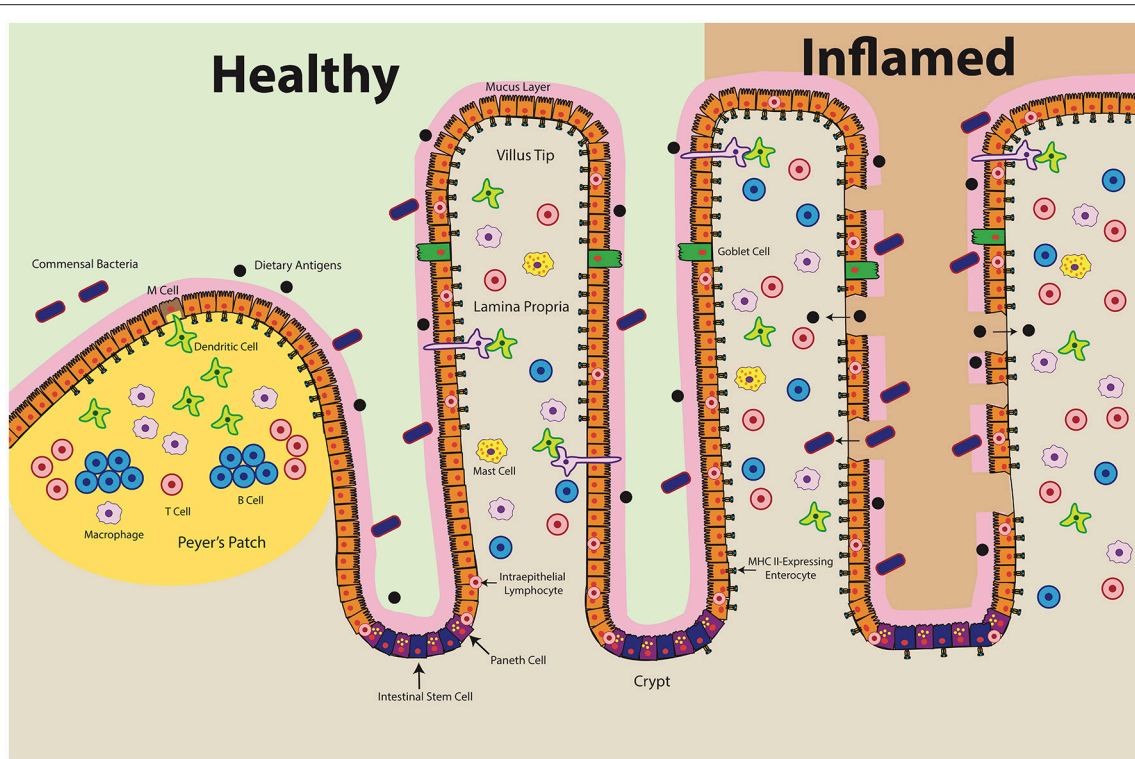
**FIGURE 2 |** Intestinal Epithelial Cell MHC Class II Expression in Health and Disease. At homeostasis (left), MHC class II is constitutively expressed in the upper villi of the small intestine. At the crypt base, intestinal stem cells self-renew and differentiate into specialized cell types: antimicrobial-producing Paneth cells, mucus-producing Goblet cells, hormone-producing enteroendocrine cells, and nutrient-absorptive enterocytes (200). Healthy crypts lack MHC class II expression. Intraepithelial lymphocytes, consisting of T cells and γδ T cells, likely play a key role in maintaining the baseline expression of MHC class II in ECs by producing IFNγ. During disease (right), MHC class II levels increase and extend into the crypts. Epithelial barrier integrity decreases, which may result in ECs encountering antigen along both the apical and basolateral surfaces. Organized lymphoid structures, known as Peyer's Patches, contain dense concentrations of professional antigen presenting cells (B cells, macrophages, dendritic cells). These cells encounter antigen delivered by microfold (M) cells, which transcytose luminal antigens. Whereas MHC class II expression has been shown in the Peyer's Patch epithelium, there are conflicting reports regarding MHC class II expression by M cells.

other reports, including a comparatively recent study, show lateral and basolateral MHC class II (73–75). These contradictory observations may be due to variability in methods of tissue processing and labeling, which has a significant effect on antigen stability and labeling efficiency (68, 76, 77). Notably, *in vitro* studies show expression of MHC class II along the basolateral surface and *in vivo* studies suggest that the amount of MHC class II along the basolateral surface of IECs is physiologically relevant (78–80).

Intestinal inflammation may also change MHC class II localization in IECs. Both conventional and electron microscopy have been used to show redistribution of IEC MHC class II from multivesicular bodies (late endosomes) to the basolateral membrane located on the submucosal side of the epithelial membrane in both celiac disease and IBD (74, 81). Increased trafficking of MHC class II to the cell surface likely requires downregulation of MARCH8 ubiquitin ligase, which drives MHC class II internalization and which IECs express at high levels (82). A similar pathway has been observed in DCs, where MARCH 1 is downregulated upon maturation stimulated by TLR ligands

(83). Redistribution of MHC class II may allow IECs to influence immune responses during a pathogenic or inflammatory insult, by presenting peptides that promote immune clearance or induce tolerance.

Co-stimulatory molecules CD80 and CD86 are not expressed on IECs at baseline (57, 84, 85). Whether these molecules are expressed during inflammation is less clear. Some studies report that human IECs express neither CD80 nor CD86 during IBD, while others show selective expression of CD86 during active disease in biopsy specimens or with IFNγ-treatment in culture (85, 86). There is also evidence that the costimulatory molecule CD40, which interacts with CD40 ligand (CD40L) on T cells, is expressed by IECs during IBD in regions with visible pathology (87, 88). IECs may provide other forms of co-stimulation, such as CD58 (LFA-3), which interacts with CD2 on the surface of T cells (89). IECs express basolateral CD58 constitutively on surgically resected colonic epithelium and *in vitro* treatment with anti-CD58 antibody inhibits stimulation of antigen-specific CD4$^+$ T cell clones by antigen-pulsed IECs in a dose-dependent manner in humans (90).

### Lung

Unlike the gut during ontogeny, fetal lung tissue does not appear to express MHC class II on AEC surfaces during gestation except in the case of active inflammation (91). Interestingly, invariant chain expression without co-expression of MHC class II has been detected on fetal alveolar epithelium by 12–14 weeks' gestational age in humans (92). Adult AECs, like small intestinal epithelium, were initially shown to constitutively express MHC class II on both bronchial and alveolar epithelium, specifically on type II pneumocytes and ciliated ECs (**Figure 3**) (93–95). However, additional studies utilizing clinical specimens have provided conflicting data, especially in primary bronchial EC cultures (96–99). Evidence in studies comparing germ-free to conventional rats supports constitutive surface expression of MHC II in lung parenchymal AECs, specifically Type II pneumocytes, but decreased expression in bronchial epithelium of germ-free rats, suggesting site-specific expression (100). Lung tissue obtained from patients with allergy or autoimmunity, including chronic bronchitis, asthma, idiopathic pulmonary fibrosis or lung transplant rejection, shows enhanced expression of MHC class II on AECs (96, 97, 101–103). Viral infection, including parainfluenza, have demonstrably increased AEC MHC class II expression, whereas bacterial infection appears to have the opposite effect in human lung specimens (91, 97, 104).
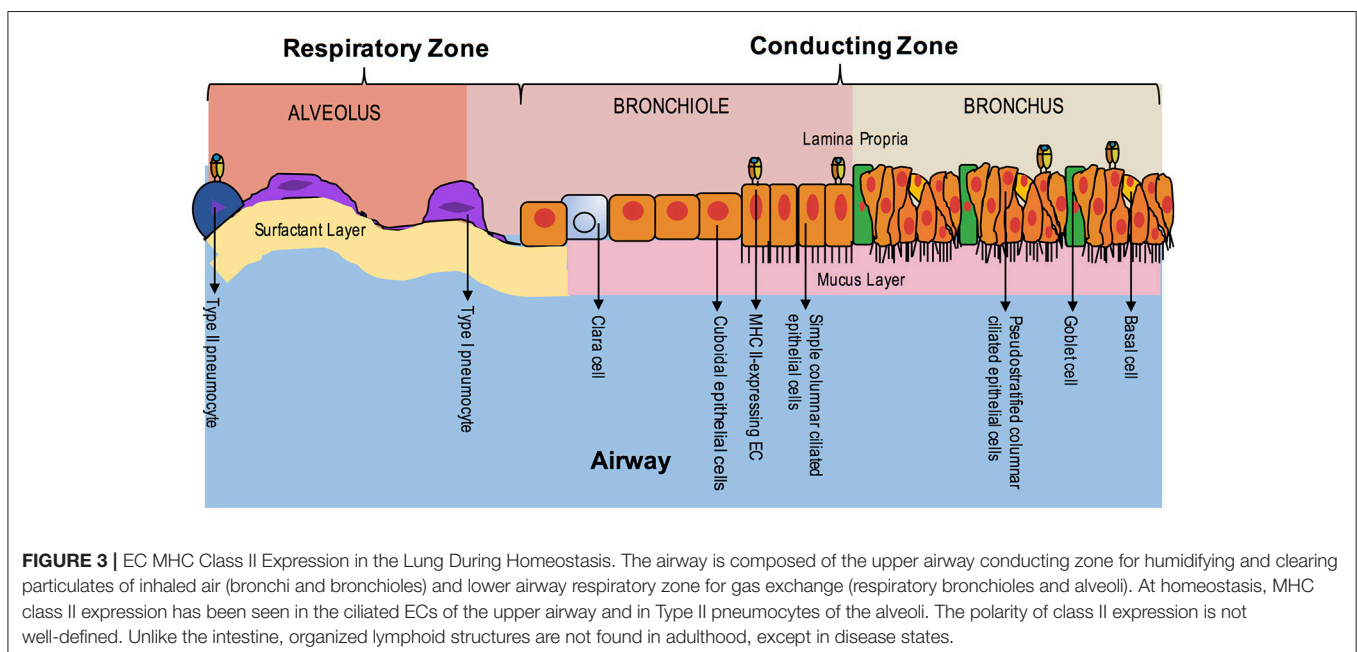
Co-stimulatory molecule expression appears to be region-specific in humans, as well. *in vitro* studies show baseline expression of CD86 on both bronchial and alveolar cells (A549 cell line), but baseline CD80 expression only on alveolar cells (98). Viral infection, specifically with rhinovirus, upregulates CD80 on alveolar cells and CD86 on bronchial cells (98). *In vivo* data obtained from lung biopsies in patients with a variety of autoimmune pathologies, including lung transplant rejection and idiopathic pulmonary fibrosis, shows increased expression of CD80 and CD86 on AEC from all segments of the respiratory

tract (97, 105). In comparison, in bronchiolitis obliterans organizing pneumonia (now known as cryptogenic organizing pneumonia), an idiopathic interstitial lung disease believed to be secondary to epithelial damage, CD80 is upregulated in AECs without concurrent upregulation of CD86 or MHC class II expression (97, 106). Like gut, CD58 is constitutively expressed on alveolar ECs, though expression has not been demonstrated in isolated Type II pneumocytes (95).

## *In vitro* Evidence for ECs as Antigen Presenting Cells

Studies utilizing human IEC lines (T84 and HT29) show that IFNγ-treated, protein antigen-pulsed IECs can stimulate antigen-specific immune responses in T cell hybridomas (107). T cell hybridomas do not need co-stimulation, which arguably mimics the reduced costimulatory requirements of the majority of T cells in the lamina propria, which are antigen-experienced memory cells (108, 109). Follow-up work in IECs found that generation of specific MHC II-restricted peptide epitopes differed if antigen was taken up from the apical or basolateral IEC surface (78). During disease, inflammatory signals including IFNγ and TNFα in the gut increase epithelial permeability (**Figure 2**) (110–112). When the epithelium is breached, IECs may interact with antigen along both the apical and basolateral surfaces, raising the possibility that novel peptide epitopes can be generated. Dotan et al. found that IECs isolated from surgically resected colon of Crohn's disease and ulcerative colitis patients induced CD4+ T cells to proliferate and secrete more IFNγ than control IECs in a mixed lymphocyte reaction (65). This effect was blocked with an anti-HLA-DR antibody.

Another mechanism by which ECs may modulate antigen presentation is through exosomes. Exosomes, cell-derived vesicles laden with MHC class II, are released extracellularly when the limiting membrane of a multi-vesicular endosome



**FIGURE 3 |** EC MHC Class II Expression in the Lung During Homeostasis. The airway is composed of the upper airway conducting zone for humidifying and clearing particulates of inhaled air (bronchi and bronchioles) and lower airway respiratory zone for gas exchange (respiratory bronchioles and alveoli). At homeostasis, MHC class II expression has been seen in the ciliated ECs of the upper airway and in Type II pneumocytes of the alveoli. The polarity of class II expression is not well-defined. Unlike the intestine, organized lymphoid structures are not found in adulthood, except in disease states.

fuses with the plasma membrane (113). In fact, several studies show that exosomes from IFNγ-treated IECs express elevated MHC class II (114–116). These exosomes express late-endosomal markers, consistent with their origin in multi-vesicular bodies. Evidence suggests that IEC exosomes do not directly stimulate antigen-specific T cells but are first acquired by DCs. DCs primed with IEC exosomes require lower doses of antigen to stimulate T cell hybridomas (116). Exosomal transfer of peptide/MHC II complexes may promote rapid, primary adaptive immune responses by equipping DCs to stimulate naive T cells. Defining the relative contributions of direct IEC antigen presentation vs. exosome release to intestinal tolerance and immunity will require further investigation and may provide important insights into the communication between gut and lung.

AECs isolated from bronchial epithelium in humans and cultured with IFNγ have been shown to trigger proliferation of allogeneic lymphocytes as well (99). Additionally, investigations by Cunningham et al. demonstrated that the addition of anti-CD28 antibody as a co-stimulatory signal allows allogeneic CD4$^+$ T cells to proliferate in response to IFNγ-stimulated MHC class II$^+$ AECs (117). Further characterization by other groups shows that purified allogeneic T cells are stimulated in response to bronchial ECs, which is abolished by the addition of anti-DR antibody (118). Bronchial ECs have also been shown to present protein antigens to antigen-specific sensitized T cells, suggesting the ability of AECs to process and present foreign antigen to the underlying lymphoid tissue (119). Experiments utilizing electron microscopy verify that AECs stimulated with IFNγ are able to endocytose antigen and that, like IECs, uptake is polarized on the luminal side of tissue explant cultures. Co-localization studies further demonstrate the trafficking of these antigens through early and late endosomes to acid vesicles and lysosomes (120).

*In vitro* studies have important caveats. MHC class II is only expressed in the large intestine and potentially bronchi during disease, yet many commonly used intestinal cell lines (Caco-2, T84, and HT29) are colon-derived and pulmonary cell lines (BEAS-2B) bronchial in origin. Therefore, studies using these cell lines may be more representative of EC antigen presentation during inflammation rather than homeostasis. Colorectal cancer cells are also susceptible to genetic and epigenetic abnormalities, including changes in DNA methylation that affect CIITA expression (121). Small intestinal EC lines, such as HEC-6 and H4, exist, but are derived from fetal tissue and are more representative of crypt stem cells than fully differentiated ECs (122). Additionally, AECs are often derived from bronchoalveolar lavage brushings or fluid in patients with additional underlying pathologies, which are highly operator- and patient-dependent and may not be representative of the entire airway epithelium. Furthermore, *in vitro* experiments using peripheral blood T cells may not recapitulate interactions between ECs and organ-specific T cells. For instance, one study shows that IECs induce CD4$^+$ IELs to secrete IFNγ, but not CD4$^+$ T cells from the lamina propria or spleen (123). Moreover, CD4$^+$ and CD8$^+$ T cells found in adult human mucosa, including in both gut and lung, are largely memory cells, requiring different stimuli than naïve cells (124). Therefore, the complexity of the epithelium and the arrangements of the many cell types found within may not be well-represented in cultures of primary purified cell lines.

## *In vivo* Evidence for ECs as Antigen Presenting Cells

Several *in vivo* studies of IEC antigen presentation have focused on IBD, where inflammatory responses to the gut microbiota are believed to elicit tissue damage, yet the role of IECs themselves remain poorly defined. Maggio-Price et al. induced colonic inflammation in RAG2$^{-/-}$ mice exclusively expressing MHC class II either on IECs or DCs. Animals with MHC class II$^+$ DCs developed severe colitis, whereas mice with MHC class II on IECs developed only mild inflammation (125). Additionally, mice lacking MHC class II on DCs appeared to develop intestinal inflammation due to lack of proper CD4$^+$ T cell-mediated adaptive immune responses to commensal bacteria, as gnotobiotic mice under the same conditions did not develop inflammation (126). In a different murine colitis model, Thelemann et al. showed that selectively knocking out MHC class II in IECs worsened colitis; additionally, mice without IEC MHC class II had higher IFNγ levels and a reduced proportion of Tregs (80). In this system, IECs failed to express CD40, CD80, or CD86 co-stimulatory molecules. Another elegant study targeted hemagglutinin (HA) expression to IECs in transgenic mice expressing an HA-specific T cell receptor. This resulted in the expansion of HA-specific Tregs and was not dependent on DCs acquiring antigen from apoptotic IECs. Isolated primary IECs directly stimulated Treg proliferation in an MHC class II-dependent manner (79). Interestingly, the authors ruled out TGF-β and retinoic acid as effectors—molecules known to skew naive T cells into induced Tregs (127). Together, this suggests a tolerogenic role for IECs that is not dependent on co-stimulation of CD80 or CD86. Similar *in vivo* data has not been collected in the respiratory tract of animal models, and effects on the lung epithelium were not evaluated in the above models.

## Cytokine Regulation

Potential immune cell sources of IFNγ that upregulate MHC class II include natural killer (NK) and natural killer T (NKT) cells, group 1 ILCs (ILC1s), γδ T cells, CD8$^+$ T cells and subsets of CD4$^+$ T cells, which comprise the makeup of the GALT (128–132). The pIV isoform of the MHC class II transactivator CIITA is the main form expressed in non-hematopoietic cells in response to interferon gamma (IFNγ) and has been found in IECs (133, 134). Adoptive transfer of CD4$^+$ T cells into mice induces IEC MHC class II expression, whereas the transfer of IFNγ-knockout T cells does not (80). Direct treatment with IFNγ has been shown to increase AEC MHC II expression in rats both *in vitro* and *in vivo* (100, 119). These findings have been re-capitulated in human AECs *in vivo*, as well (99, 135). AECs may enhance MHC class II expression via a CIITA-independent pathway, at least when exposed to viral particles, though data appear conflicting (104, 136). A possible explanation for tissue-specific differences in cytokine regulation may be that IELs or other cell types that produce IFNγ to drive MHC class II in IECs, such as NK cells or ILC1, may be more abundant in the GALT compared to the airway, though further characterization is required (137–139).

To date, there is limited evidence that cytokines besides IFNγ induce MHC class II on non-hematopoietic cells during inflammation. One candidate is IL-27, an IL-12 superfamily cytokine released by activated DCs and elevated during intestinal inflammation (140). IL-27 elevates CIITA levels in colorectal cancer cells and stimulates endothelial cells *in vitro* to express HLA-DR, -DP, -DQ, -DM, and invariant chain (141, 142). The overlapping effects of IL-27 and IFNγ are consistent with the fact that both cytokines promote T helper 1 (Th1) CD4^{+} T cell responses (143).

Another potential candidate is IL-18. IL-18, originally known as IFNγ inducing factor or IGIF, is a member of the IL-1 cytokine family and can mediate both Th1- and Th2-type responses (144–147). IL-18 has been shown to be elevated in autoimmune colitis including IBD and celiac disease and is a key mediator of intestinal homeostasis (144, 148–152). IEC appear to constitutively produce IL-18, at least *in vitro*, and promote increased production of IFNγ by T cells obtained from patients with active inflammation (153, 154). Interestingly, tissue explants from patients with active celiac disease show IL-18 expression only in the crypts (151). In a murine model of an NLRC4 inflammasome mutation, the intestinal epithelium appears solely responsible for the systemic elevation of IL-18 seen in macrophage activation syndrome and, furthermore, is associated with upregulation of IFNγ-induced genes and multiple genes associated with antigen presentation in the intestine (155). Recent evidence in humans shows that AEC also constitutively produce IL-18 *in vitro* in animal models (156, 157). IL-18 has been shown to directly upregulate MHC class II expression on IFNγ-stimulated keratinocytes, but this has not yet been explored in intestinal or airway epithelium (158). Whether EC-produced IL-18 is involved in paracrine MHC class II upregulation along the crypt-to-villus axis or through directing GALT-mediated IFNγ-production also remains unknown. Therefore, further investigation is needed to determine if these or other region-specific cytokines upregulate EC MHC class II expression.

### Role of the Microbiome

Commensal bacteria reside within the lumen of the gut, reaching a density of up to $10^{12}$ cells per $cm^3$ in the large intestine (159). It is well-established that these microbes contribute to the development of the intestinal immune system; gnotobiotic mice, for example, do not form isolated lymphoid structures in the small intestine (160). Though the lung and gut share a common origin at the oropharynx, microbial populations are vastly different. The lung is not completely sterile but has a much lower bacterial burden without a characteristic microbiome like the gut; rather, lung flora tends to resemble oral flora and may change in response to a variety of stimuli and pathologies (161, 162).

Significantly, IECs of gnotobiotic mice lack MHC class II expression, while exposure to bacteria is found to increase IFNγ expression by γδ T cells and induce CIITA and MHC class II (163–165). There is limited but interesting evidence that specific classes of commensals, such as segmented filamentous bacteria, are sufficient to induce MHC class II in IECs (165). However, because γδ T cells compose a higher proportion of IELs in mice (~50%) than in humans (~10%), other cellular sources of class II inducing signals may be important in humans (166, 167). Additionally, the roles of viruses and fungi within the microbiome and their effects on EC MHC class II expression remain largely unexplored.

Recent evidence argues for a reciprocal effect of MHC class II in shaping the microbiome. Studies in natural fish populations link MHC class II allelic variation with the abundance of certain microbial taxa (168). These findings corroborate studies in laboratory mice, which show that MHC class II-linked changes in the microbiome mediate risk of enteric infection and autoimmune disease, such as type 1 diabetesc (169, 170). The precise mechanisms behind these effects remain poorly understood, though there is evidence that MHC class II polymorphisms control microbial populations through IgA phenotype and thus modify susceptibility to pathogens (169).

Exploration into the "gut-lung axis" in which the microbiome of the gut has direct impact on susceptibility to pulmonary disease is of key interest (171). The gut microbiome has been shown to affect lung susceptibility to infection with viral, fungal and bacterial pathogens (172–176). The severity of ozone-induced asthma in mice appears to be regulated by the gut microbiome through short chain fatty acid production (177). Segmented filamentous bacteria, a gut commensal that induces MHC class II on IECs as above, have independently been shown to provoke autoimmunity of the lung epithelium, but whether this is through affecting MHC class II expression on AECs is unknown (165, 176). The microbiome may even affect predisposition to lung cancer as evidenced through murine studies focused on probiotic use, though further mechanistic and human studies are still needed in this area, as well (171, 178).

## DISCUSSION

Though much of the available evidence on MHC class II expression by ECs was obtained decades ago, this is an exciting time for research into the role of ECs in mucosal immunology. Renewed awareness of the role played by epithelial cells in homeostasis and disease and technical advances in different areas open up several new avenues for research and clinical applications.

Celiac disease, in which blunting of the villous tips on biopsy is pathognomonic, provides an example of a disease in which the role of the EC should be re-visited. Pathogenic CD4^{+} T cells in celiac disease are DQ2- or DQ8-restricted, and T cell bound to DQ/gliadin tetramers are detectable using flow cytometry (179–182). Yet, most MHC class II studies examined in this review focus on HLA-DR. Cell surface HLA-DP and -DQ levels have been reported as lower, but T cells are remarkably sensitive, requiring as few as one to ten peptides per MHC II complexes for activation (183). Levels of MHC class II that are below the limit of detection by immunohistochemistry (used in many early papers) may therefore be sufficient to activate T cells. More sensitive techniques, such as flow cytometry or electron microscopy, are more informative, as evidenced by more recent papers. Another novel possibility is investigation utilizing multiplexed ion beam

imaging (MIBI) to visualize large panels of cell-surface proteins tagged with elemental metals that may allow improved detection of MHC II isoforms and co-localization of various co-stimulatory molecules on tissue sections (184). Using these technologies to study celiac disease, a model disease in which the inciting immunogen and the presenting MHC class II-molecules are known, may provide important insights into the role of ECs in antigen presentation.

The function of co-stimulatory molecules in this process is another area that requires more investigation. While some description of EC surface expression of classic B7 molecules, CD80 and CD86, is found in the literature (above), their roles during homeostasis and inflammation remain unclear. The lack of expression of CD86 found in the gut, compared to constitutive expression in the airway, may suggest a diminished role of IECs in interactions with Tregs (185). Another avenue of exploration are other members of the B7 family that appear to have novel roles on non-professional antigen presenting cells, including ICOS ligand, PD-L1 (B7-H1), PD-L2 (B7-DC), B7-H3, and B7-H4. Work on the ICOS co-stimulation pathway in the airway already has provided promising results, with anti-ICOS treatment leading to prevention of chronic lung transplant rejection and obliterative bronchiolitis as well as ICOS being shown as an important player in asthma (186, 187). However, the contribution of the aerodigestive epithelium in mediating these interactions remains to be explored.

Further delineation of the subsets and character of ECs are needed as well. The epithelium is composed of both stem cells and specialized subtypes as described above, many of which remain poorly understood. Both MHC II expression and antigen presenting capabilities and function may therefore differ among these cells. Work reviewed here has shown that, for example, M cells in the gut or type II pneumocytes in the lung may have roles in antigen presentation and expression of HLA-DR (9, 11, 16). Furthermore, the polarity and anatomic localization of intestinal and pulmonary ECs also likely bear significant implications for antigen uptake, processing and presentation and warrant further investigation (53, 55, 71–73, 78–80). Defining the roles of these various cell types and their locoregional interactions thus may provide additional important insights.

The conditions under which mucosal MHC class II contributes to inflammation vs. tolerance also remain to be clearly delineated. The work by Westendorf et al. in mouse models shows direct expansion of Tregs in response to ECs, arguing that mucosal MHC class II can function in a tolerogenic role, though the work by Dotan suggests that increased MHC class II on IECs from IBD patients may more efficiently activate effector T cell responses leading to inflammation (65, 79, 188). Moreover, it is plausible that MHC class II on ECs not only allows ECs to modulate immune responses, but also in fact allows the immune system to regulate the epithelium. Cytokines released by adjacent mucosal and intraepithelial immune cells in response to EC presentation of MHC class II-bound peptides may alter cell renewal, barrier integrity, cell type composition, and the innate immune functions of the epithelium. A promising approach to explore these questions is in organoids derived from stem cells or induced pluripotent stem cells that can differentiate into specialized cell types mimicking the physiological structure of the epithelium (189–192). Organoids offer a reductionist setting for testing the role of immune cells, cytokines, pathogens, and other regulatory factors on MHC class II in primary ECs in a way that appears to model organs physiologically. For example, mouse intestinal organoids treated with IFN have been shown to upregulate MHC class II (193). Intestinal organoids can be readily infected with human strains of enteric pathogens, such as rotavirus, norovirus, and *Salmonella* to allow exploration of MHC class II internalization and polarity during infection and inflammation (194–196). The study of lung organoids remains, comparatively, in its infancy, though work has been done to create structures resembling fetal lung buds in the second trimester of gestation for the study of respiratory syncytial virus (197). Organoids may provide a model system to study the aforementioned hypotheses to provide evidence more pertinent to humans.

Finally, current research is actively exploring the contributions of the microbiome to systemic immunity. However, how the microbiome changes EC structure and function, especially through MHC class II and co-stimulatory molecule expression, and whether this affects development of disease and ultimate outcomes are also key questions. Highlighting the importance of co-stimulatory molecules, recent clinical work has demonstrated that the efficacy of cancer immunotherapies targeting B7 molecules PD-L1 or CTLA-4 in epithelial cancers including non-small cell lung carcinoma as well as colon cancer appear keenly dependent on the gut microbiome; lack of or depletion of commensals using oral antibiotics appears to attenuate tumor response to these therapies (198, 199). Interestingly, blockade of CTLA-4 on IELs led to IEC apoptosis in intestinal organoids, also suggesting a bidirectional trophic communication between ECs and effector immune cells through co-stimulatory molecules (199). Valuable lessons may be learned by comparing MHC class II expression on ECs in the intestinal and respiratory tracts.

Ultimately, systems that integrate immunological, microbial, and environmental signals to study EC MHC class II expression and function are likely to advance our understanding of mucosal immunity and the epithelium of the aerodigestive tract. How these findings can be manipulated to affect infectious, autoimmune or even neoplastic diseases will likely be pursued in the coming years.

## AUTHOR CONTRIBUTIONS

JW, DM, and EM were involved in planning the project. JW, DM, CM, and EM contributed to the writing of the final manuscript.

## ACKNOWLEDGMENTS

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