

## Review Article



# Recent Insights into Cellular Crosstalk in Respiratory and Gastrointestinal Mucosal Immune Systems

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## OPEN ACCESS

Received: Aug 29, 2020

Revised: Oct 16, 2020

Accepted: Oct 18, 2020

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### Conflict of Interest

The authors declare no potential conflicts of interest.

### Abbreviations

ACE2, angiotensin-converting enzyme 2; AM, alveolar macrophage; AT, alveolar type; C5aR, complement C5a receptor; CBC, crypt base columnar; cDC, conventional dendritic cell; CLN, cervical lymph node; COVID-19, coronavirus disease 2019; CSF2, colony-stimulating factor 2; CSR, class switch

## ABSTRACT

The human body is continuously threatened by pathogens, and the immune system must maintain a balance between fighting infection and becoming over-activated. Mucosal surfaces cover several anatomically diverse organs throughout the body, such as the respiratory and gastrointestinal tracts, and are directly exposed to the external environment. Various pathogens invade the body through mucosal surfaces, making the mucosa the frontline of immune defense. The immune systems of various mucosal tissues display distinctive features that reflect the tissues' anatomical and functional characteristics. This review discusses the cellular components that constitute the respiratory and gastrointestinal tracts; in particular, it highlights the complex interactions between epithelial and immune cells to induce Ag-specific immune responses in the lung and gut. This information on mucosal immunity may facilitate understanding of the defense mechanisms against infectious agents that invade mucosal surfaces, such as severe acute respiratory syndrome coronavirus 2, and provide insight into effective vaccine development.

**Keywords:** Gastrointestinal tract; Infection; Mucosal immunity; Respiratory tract

## INTRODUCTION

The mucosal immune system consists of immune inductive and effector sites (1). Mucosal immune inductive sites include mucosa-associated lymphoid tissues (MALTs) and mucosa-draining lymph nodes (LNs). Examples of LNs include the mesenteric (MLNs) and cervical LNs (CLNs). MALTs are solitary organized structures containing T and B cell follicles; the gut-associated lymphoid tissue (GALT) and nasopharynx-associated lymphoid tissue (NALT) are typical examples of MALTs (Fig. 1). The lamina propria (LP) of the mucosa, which is an immune effector site, is situated within the subepithelial stroma of mucosal connective tissue and contains effector T cells, plasma cells, macrophages, and dendritic cells (DCs).

IgAs are humoral immune effectors, of which secretory IgA (SIgA) is a primary humoral immune component of the mucosa (2). IgA, which is found in the LP, is produced by plasma cells in dimeric form, whereby two IgA components are linked together by a joining chain. The dimerization of IgA is necessary for its secretion, which is facilitated by the polymeric

### Respiratory tract

#### Upper respiratory tract

Nasal cavity

Pharynx

#### Lower respiratory tract

Trachea

Bronchus

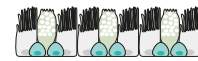
Bronchioles

Alveoli

Lung

Alveoli

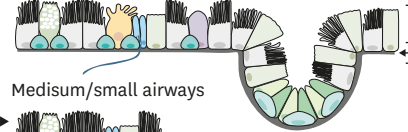
Nasal epithelial cells



Nasopharynx-associated lymphoid tissue (NALT)



Cartilage/bronchus

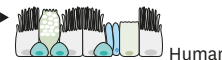


Pseudostratified epithelium

Basement membrane

Submucosal gland

Mediastinum/small airways

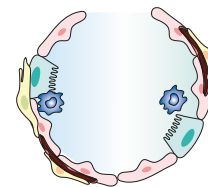


Human



Mouse

Alveolus



### Gastrointestinal tract

#### Mesenteric lymph nodes

#### GALT

- Isolated lymphoid follicles

- Peyer's patches

Caecum

Stomach

Duodenum

Jejunum

Ileum

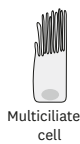
Colon with caecum & appendix

Jejunum

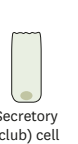
Ileum

Colon

Peyer's patch



Multiciliated cell



Secretory (club) cell



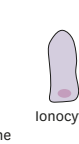
Goblet cell



Brush (tuft) cell



Pulmonary neuroendocrine cell (PNEC)



Ionocyte



Basal cell



Serous cell



Mucous cell



Myoepithelial cell



Alveolar type 1 (AT1) cell



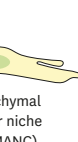
Alveolar type 2 (AT2) cell



Microfold (M) cell



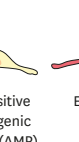
Mesenchymal alveolar niche cell (MANC)



Axin2-positive myofibroblastic precursor (AMP)



Endothelial cell



Columnar epithelial cell



Paneth cell



Crypt base columnar cell



Absorptive progenitor cell



Secretory progenitor cell



Enteroendocrine cell

**Figure 1.** Cellular composition of the respiratory and gastrointestinal tracts. (A) The respiratory tract consists of the upper (nasal cavity, pharynx) and lower (trachea, bronchus, bronchioles, alveoli) tracts. They are covered by a single layer of epithelial cells, such as multiciliated cells, club cells, goblet cells, basal cells, PNECs, tuft cells, ionocytes, serous cells, myoepithelial cells, AT1 and AT2 cells, mesenchymal alveolar niche cells, and AMPs. (B) The small and large intestines of the gastrointestinal tract are distinguished by the crypt-villus structure and are composed of columnar epithelial, microfold, Paneth, goblet, enteroendocrine, and tuft cells. AMP, axin2-positive myofibroblastic precursor.

recombination; DC, dendritic cell; FAE, follicle-associated epithelium; FcRn, neonatal Fc receptor; FDC, follicular dendritic cell; GALT, gut-associated lymphoid tissue; GC, germinal center; GP2, glycosylphosphatidylinositol-anchor protein 2; 5-HT, 5-hydroxytryptamine; IEC, intestinal epithelial cell; IFR, interfollicular region; ILC2, type 2 innate lymphoid cell; LN, lymph node; LP, lamina propria; M, microfold; MALT, mucosa-associated lymphoid tissue; MLN, mesenteric lymph node; NALT, nasopharynx-associated lymphoid tissue; pIgR, polymeric Ig receptor; PNEC, pulmonary neuroendocrine cell; PP, Peyer's patch; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SED, subepithelial dome; SIgA, secretory IgA; SIgM, secretory IgM; SILT, solitary isolated lymphoid tissue; SMG, submucosal gland; SP, surfactant protein; Tfh, follicular helper T; TMPRSS2, type II transmembrane serine protease.

#### Author Contributions

Conceptualization: Jang YS, Kim SH. Data curation: Kim SH, Jang YS. Formal analysis: Jang YS, Kim SH. Funding acquisition: Jang YS. Investigation: Kim SH, Jang YS. Project administration: Jang YS. Supervision: Jang YS. Validation: Jang YS. Visualization: Kim SH, Jang YS. Writing - original draft: Kim SH. Writing - review & editing: Jang YS.

Ig receptor (pIgR); the secretory component of pIgR covalently interacts with dimeric IgA, completing the structure of the SIgA complex (3). The SIgA complex is transported by pIgR from the basolateral to the apical side of the membrane, then released from pIgR, and secreted to the mucosal surface. SIgA complexes are protected from proteolysis by N-glycosylation of the secretory component.

Alongside SIgA, secretory IgM (SIgM) is also present in mucosal secretions (4). Pentameric IgM is translocated to the apical side of the mucosal membrane following binding to pIgR via the joining chain. IgG levels are notably higher than SIgA levels in the lower respiratory lumen of human airways (5). IgG can be transported into the lumen via the neonatal Fc receptor (FcRn), which comprises MHC class I-like transmembrane protein and  $\beta 2$  microglobulin (6). FcRn also functions as an apical transporter. Although secretory IgD is present in mucosal secretions within aerodigestive mucosa, the basolateral transport mechanism for IgD is unclear (7). These Igs maintain mucosal homeostasis by controlling commensals and protecting against pathogens (8).

The secretory mechanisms of humoral effectors described above are common across various mucosal immune compartments. However, the immune systems of diverse mucosal tissues also exhibit unique characteristics reflecting their anatomical functions. In this review, we discuss the diverse characteristics of regional cellular components in the mucosal immune system of the respiratory and gastrointestinal tracts. In particular, we focus on the mucosal immune response in the humoral defense network of the lung and gut.

## CELLULAR COMPONENTS OF THE RESPIRATORY AND GASTROINTESTINAL TRACTS

Respiratory and gastrointestinal epithelia exhibit distinct characteristics but likewise share some anatomical similarities. Both epithelia are lined with columnar epithelial cells with cilia (respiratory tract) or microvilli (gastrointestinal tract), which they produce a physical barrier between the inner body and the environment and act as sentinels for the immune system.

### Cellular components of the respiratory system

Respiratory tissues span multiple organs, from the upper respiratory tract (nostrils, nasal cavity, and pharynx) to the lower respiratory tract (trachea, bronchi, bronchioles, and alveoli) (Fig. 1A) (9). The respiratory tract is wrapped with bands of smooth muscle, which supports to airway flow. In humans, a pseudostratified epithelium covers the trachea up to the distal bronchioles; however, in mice, the pseudostratified epithelium extends only to the distal intralobar bronchi (10). The pseudostratified epithelium is a single layer of epithelial cells comprising mainly multiciliated cells, club cells, goblet cells, and basal cells. Multiciliated cells have hundreds of motile cilia on their apical area and transport inhaled particulates and mucus out of the airways retrogradely (11). Club cells, with their dome-shaped apical surface, are primary secretory cells that specifically express uteroglobin (secretoglobin family 1A member 1), the most abundant protein in mucosal lung secretions, and contribute to homeostatic maintenance of the airways (12). Goblet cells secrete mucus, which blocks the influx of inhaled particulates. The secretion of mucus is regulated by IL-13, which is produced by type 2 innate lymphoid cells (ILC2s). Basal cells act as resident stem cells that are capable of long-term self-renewal and differentiation into basal, ciliated, and club cells during homeostasis and after injury (13). The fate of regenerated cells depends on Notch signaling (14).

The pseudostratified epithelium contains uncommon cell types, including pulmonary neuroendocrine cells (PNECs), tuft cells, and ionocytes (15). PNECs comprise approximately 1% of the airway epithelium, where they are the only innervated cell type. They manifest in clusters known as neuroepithelial bodies in mouse lungs. PNECs function as sensory cells by secreting neuropeptides (e.g., calcitonin gene-related peptide), neurotransmitters (e.g., serotonin), and bombesin-related peptides (e.g., neuromedin B) in response to changes in oxygen levels (16). PNECs also serve as a modulator in the immune responses to allergens and in tissue remodeling (16). Tuft cells are referred to by different names, depending on the organs in which they are present: brush cells in the airway, microvillous cells in the nasopharyngeal cavity, and multivesicular caveolated cells in the intestine (17). Tuft cells found in the lung specifically express type II taste receptors and bitter taste receptors; activation of the former promotes the release of antimicrobial peptides from neighboring ciliated cells by modulating  $\text{Ca}^{2+}$  influx. Ionocytes are major expressers of cystic fibrosis transmembrane conductor regulator and contribute to the regulation of mucus production (18). Submucosal glands and cartilaginous rings control airway function in the lower respiratory tract (15). Various cell types line the submucosal glands, including ciliated, goblet, serous, and myoepithelial cells; these cells secrete mucus and host defense proteins in response to pathogens and toxic substances.

The distal region of the small airways contains the alveolar sacs, where oxygen–carbon dioxide exchange occurs, and is hence referred to as the respiratory zone. The alveolar epithelium comprises two major epithelial lineages: alveolar type (AT) 1 and 2 cells. AT1 cells are thin and elongated and cover >95% of the alveolar surface of the adult lung (19). AT1 cells are associated with pulmonary capillary epithelial cells and mesenchymal cells to form a thin, diffusible gas exchange interface. AT2 cells are cuboidal epithelial cells and can be distinguished by their lipid-rich lamellar bodies containing surfactant proteins (SPs), such as ATP-binding cassette class A3 and SPs A–D (20). SPs reduce the surface tension of the epithelium and prevent atelectasis during respiration. AT2 cells act as alveolar epithelial progenitors when they detect the Wnt signal released from fibroblasts; in a steady-state condition, Wnt-responsive AT2 cells can self-renew and differentiate into AT1 cells, a process that is regulated by bone morphogenetic protein signaling (21). The alveolar structure is supported by the extracellular matrix produced from interstitial fibroblasts, such as Axin2-positive myogenic precursors, Wnt2-expressing platelet-derived growth factor- $\alpha$ -positive cells, and mesenchymal alveolar niche cells.

### Cellular components of the gastrointestinal system

The gastrointestinal tract has a continuous tube structure and, unlike the respiratory tract, displays regionally distinctive anatomical features, such as in the mouth, pharynx, esophagus, stomach, small intestine, large intestine, and anus (**Fig. 1B**). The gastrointestinal tract is organized with crypt-villus structures; villi are the finger-like projections found within the intestinal wall, and crypts comprise the invaginated structures between villi (22). Villi extend along the surface area of the digestively active epithelium, mainly in the small intestine. The length of the villi decreases along the small intestinal tract, and the small intestine is subdivided into three segments according to villus length: the duodenum, jejunum, and ileum, in descending order. In contrast, the large intestine has a flat epithelial surface interspaced by crypts. The large intestine is subdivided into the caecum, proximal colon, transverse colon, distal colon, and rectum, in descending order (22).

The layers of intestinal epithelial cells (IECs) can be categorized by their primary functions; columnar epithelial and microfold (M) cells are primarily involved in absorption, whereas Paneth, goblet, enteroendocrine, and tuft cells are involved in secretion. All IECs are derived from crypt base columnar (CBC) cells, known as intestinal stem cells, for which *Lgr5* is a specific marker (23). The *Lgr5*<sup>+</sup> CBC cells located inside the crypt differentiate into multiple lineages of cells and self-renew for long-term maintenance (24). Notch signals promote the differentiation of CBC cells into absorptive progenitor cells, which rapidly proliferate and mature to enterocytes and M cells, as induced by bone morphogenetic protein and receptor activator of NF- $\kappa$ B ligand signaling, respectively (25,26). Columnar epithelial cells, known as enterocytes, are the predominant cell type that absorbs digested nutrients in the small intestine. Enterocytes also play an essential role in maintaining mucosal homeostasis by regulating mucosal integrity and crosstalk among immune cells, including DCs and intraepithelial lymphocytes (27). The M cells located in the follicle-associated epithelium (FAE) of GALTs are specialized in the uptake of luminal Ags (28). M cells are recognized by their unique morphology, which constitutes short, irregular microvilli and an intraepithelial pocket containing various leukocytes (29). M cells can also be identified by monoclonal Abs specific to  $\alpha$  (1,2)-fucose-containing carbohydrate moieties or by proteins expressed specifically on the apical surface of M cells, such as glycosylphosphatidylinositol-anchor protein 2 (GP2) and complement C5a receptor (C5aR) (30,31). M cells take up luminal Ags but are also utilized as an infection route by many pathogens, such as *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Listeria monocytogenes* (29).

Secretory progenitor cells mature into Paneth, goblet, enteroendocrine, and tuft cells when they detect Wnt and other unidentified signals (23). Paneth cells are observed only in the small intestine and are concentrated mainly within the crypts of the terminal ileum. Paneth cells secrete various antimicrobial proteins, such as  $\alpha$ -defensin, lysozymes, and C-type lectins (32). Additionally, they control crypt stem cell activity by secreting epidermal growth factor and Wnt3 and by expressing Notch ligand Delta-like 4 (33). Unlike Paneth cells, the frequency of mucin-secreting goblet cells in the lower tract increases towards the colon (34). The small intestine is covered by a loose layer of mucus known as glycocalyx; the colon also contains glycocalyx, as well as a denser mucosal layer underneath. The major component of intestinal mucus is mucin 2, a lack of which induces spontaneous colitis by allowing direct contact of IECs with bacteria (35). Another secretory product of goblet cell is small protease-resistant trefoil factor 3, which binds to the cysteine-rich domain of mucin 2 and influences the viscosity of mucus (36).

Hormone-producing enteroendocrine cells are distributed throughout the gut mucosa in the crypts and villi, but they only constitute 1% of the total gut epithelial cell population (37). Enteroendocrine cells comprise several subgroups showing regional differences, and their primary function is to orchestrate responses to ingested nutrients by secreting individual hormone peptides in the gut mucosa (38). I and K cells present in the jejunum secrete cholecystokinin and gastric inhibitory peptide, respectively, along with 5-hydroxytryptamine (5-HT, serotonin). L cells are present in the ileum and colon, where they secrete glucagon-like peptides 1 and 2 and polypeptide YY. Enterochromaffin cells, the most abundant enteroendocrine cell type, are distributed throughout both the small and large intestines and secrete 5-HT. Enteroendocrine cells play an essential role in gut sensing via the expression of various receptors, such as G protein-coupled receptors 40, 41, and 43, TLRs 1, 2, and 4, and taste receptors (types I and II). Tuft cells comprise approximately 0.4% of IECs and are characterized by their bottle-shaped morphology, apical microvilli, and the expression of



transient receptor potential cation channel subfamily M member 5 (39). Tuft cells are the sole producers of IL-25, which promotes type 2 immune responses and intestinal remodeling via the activation of ILC2s (40). Collectively, these epithelial cells actively produce a mucosal barrier to block the invasion of luminal Ags and pathogens.

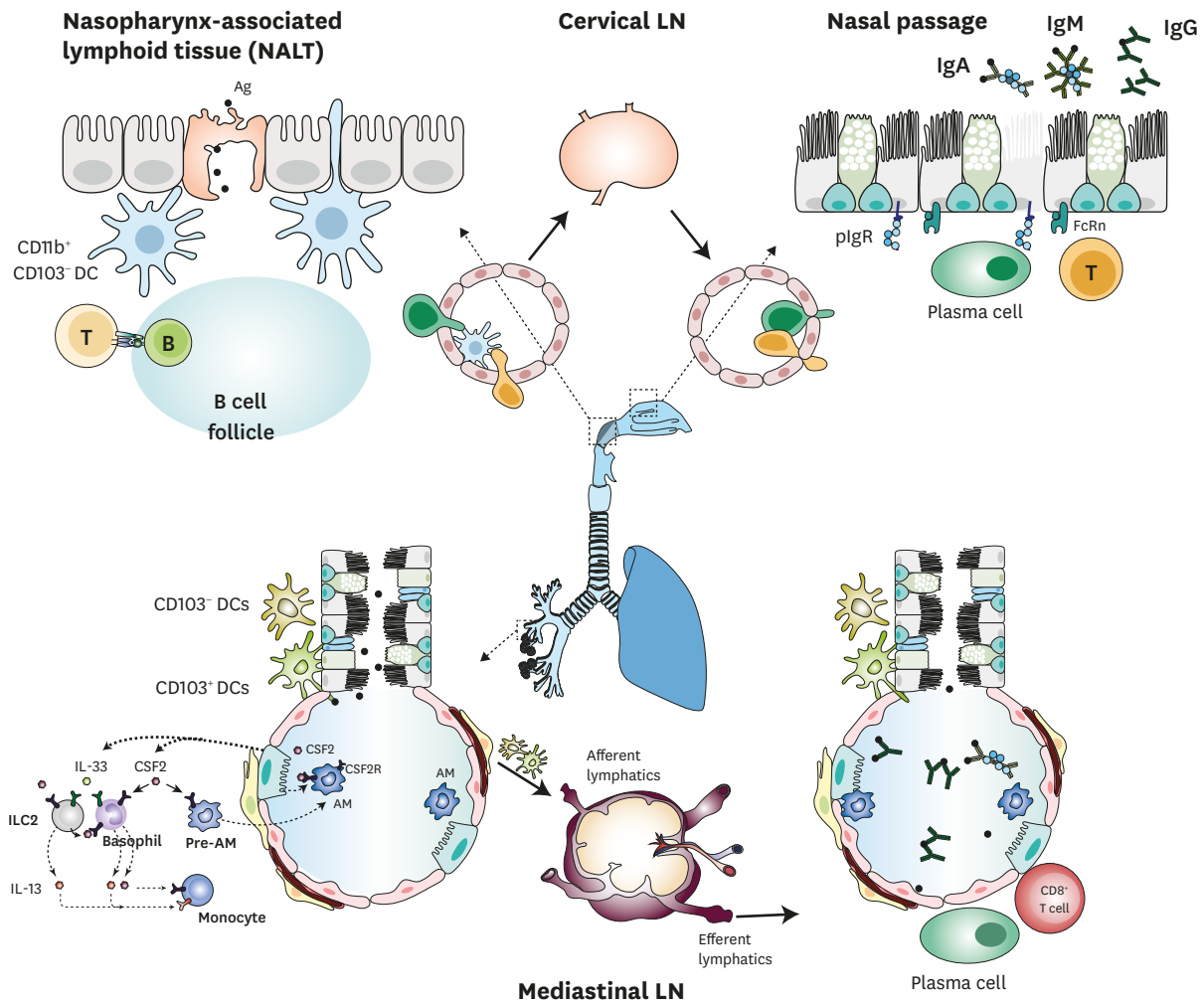
### Cellular crosstalk between epithelial cells and immune cells in the lung and gut

Under steady-state conditions, AT2 cells are the primary source of colony-stimulating factor 2 (CSF2) and IL-33 in the lung, which play critical roles in lung-specific imprinting of pulmonary basophils and in stimulating ILC2s to produce CSF2 and IL-13 (41). CSF2 is a critical modulator of differentiation and/or maturation of alveolar macrophages (AMs) from fetal liver embryonic precursors or immature AMs (42). AMs reside in the alveolar lumen, where they clear surfactants and act as immune modulators. CSF2 signaling regulates tissue-specific differentiation of AMs by inducing the master transcription factor peroxisome proliferator-activated receptor gamma, which is a key regulator of lipid metabolism (43). AMs express TGF- $\beta$ , which prevents unnecessary immune activation (44). Human AMs share common surface markers with lung macrophages, such as HLA-DR, CD11b, CD11c, and CD64; they can be distinguished from lung macrophages by examining autofluorescence and detecting the expression of CD206, CD169, and MARCO (45). Interstitial macrophages reside in the space between the lung epithelium and capillaries. They consist of two main populations: LYVE-1<sup>low</sup>MHC II<sup>high</sup> and LYVE-1<sup>high</sup>MHC II<sup>low</sup> cells. LYVE-1<sup>low</sup>MHC II<sup>high</sup> cells are located close to neurons and are specialized in Ag presentation (46). LYVE-1<sup>high</sup>MHC II<sup>low</sup> perivascular macrophages are functionally involved in wound healing and tissue repair.

Under steady-state conditions, myeloid cells in the small intestine express IL-1 $\beta$ , IL-6, and IL-23 (47). The adhesion of segmented filamentous bacteria to IECs induces the release of serum amyloid A, which triggers the expression of IL-1 $\beta$  and IL-23 in DCs (48). These cytokines promote Th17 cell differentiation and activation of group 3 innate lymphoid cells. Regenerating islet-derived protein 3 $\gamma$  also promotes IEC repair (49). Mucin production in goblet cells is increased via the IL-22-signal transducer and activator of transcription 3 axis (50). Tuft cells constitutively express IL-25, which induces ILC2 activation, resulting in IL-5, IL-9, and IL-13 secretion. IL-13 triggers the differentiation of epithelial cells into goblet and tuft cells, causing a feedback loop (40). In the large intestine, enteroendocrine L cells express glucagon-like peptide-2, which inhibits colonic crypt cell apoptosis and increases crypt depth and colon length (51). In addition, bacterial metabolites, including butyrate, trigger the release of 5-HT, which plays critical roles in enteric nervous system development, gut motility, T cell activation, and eosinophil migration (52).

## INDUCTION AND REGULATION OF MUCOSAL IMMUNE RESPONSES IN THE RESPIRATORY SYSTEM

IgA is the most abundant Ab in the upper respiratory tract, whereas IgG is dominant in the lower respiratory tract (53). NALT is a mucosal immune inductive site in the upper respiratory tract that is composed of several cell types and structures, including FAE cells, M cells, B cell follicles containing uncommitted (IgD<sup>+</sup> and IgM<sup>+</sup>) B cells, T cell areas with DC enrichment, and high endothelial venules (54). Following initial Ag exposure in NALT, the germinal center (GC) is induced; nasal DCs take up Ags and migrate into CLNs, which are also immune inductive sites (Fig. 2). Nasal DCs encounter Ags via their dendrites or M cells, which are specialized epithelial cells that take up luminal Ags via pinocytosis and endocytosis. Effector



**Figure 2.** Induction of mucosal immune responses in the respiratory tract. In the NALT, nasal DCs take up Ags via M cells and their dendrites, and then migrate to B cell follicles and CLNs. Ag-specific effector cells are recruited to the nasal passage, where they secrete Ag-specific IgG, IgA, and IgM. In the lower respiratory tract, Ag-stimulated DCs migrate to mediastinal LNs and initiate the Ag-specific immune response via activation of naïve T cells. Effector cells then home to alveolar sacs.

cells, including Ag-specific IgA<sup>+</sup> B cells, move to the nasal passage, which is a mucosal effector site. The nasal passage epithelium consists of multiciliated cells and mucus-producing goblet cells (55). In the nasal passage, mucosal Abs, including SIgA and IgG, are secreted via specific receptors, pIgR and FcRn, respectively. IgM<sup>-</sup>IgD<sup>+</sup> plasma cells undergo IgM-to-IgD class switch recombination (CSR) in NALT; this process is rare or absent in systemic lymphoid tissues (8,56).

Under steady-state conditions, murine nasal DCs consist of CD11c<sup>+</sup>MHCII<sup>+</sup>CD103<sup>+</sup>CD11b<sup>+</sup> (CD103<sup>+</sup>CD11b<sup>+</sup>), CD11c<sup>+</sup>MHCII<sup>+</sup>CD103<sup>-</sup>CD11b<sup>+</sup> (CD103<sup>-</sup>CD11b<sup>+</sup>), and CD11c<sup>+</sup>MHCII<sup>+</sup>CD103<sup>-</sup>CD11b<sup>-</sup> (CD103<sup>-</sup>CD11b<sup>-</sup>) cells. CD103<sup>+</sup>CD11b<sup>+</sup> DCs predominantly reside in the nasal passage, whereas CD103<sup>-</sup>CD11b<sup>+</sup> DCs are located mostly in the FAE of NALT (57). After nasal immunization or pathogenic infection, nasal DCs, especially CD103<sup>-</sup>CD11b<sup>+</sup> cells, rapidly migrate into CLNs and induce T cell priming (54,58). Under steady-state conditions, the DCs of human nasopharyngeal tissue are composed of both major BDCA-1<sup>+</sup> cells and minor BDCA-3<sup>high</sup> cells; reduced levels of these cells are found in patients with chronic rhinosinusitis (57).

In the lower respiratory tract, immunological events occur mainly in bronchi and bronchioles, in which the interstitium contains various immune cells such as DCs, mast cells, and ILCs. Conventional DCs (cDCs) in the lung consist of CD11b<sup>high</sup>CD103<sup>-</sup>CD11c<sup>+</sup>SIRPα<sup>+</sup> cells (cDC2) and CD11b<sup>-</sup>CD11c<sup>+</sup>Langerin<sup>+</sup>CD103<sup>+</sup> cells (cDC1), which are primed for Ag uptake due to their immature states (59). CD103<sup>+</sup> DCs located below the bronchial epithelium extend their dendrites into the airway lumen via formation of tight junctions with epithelial cells (60). Following the uptake of inhaled Ags, CD103<sup>+</sup> DCs are stimulated by alarmins, including thymic stromal lymphopoietin and IL-25. Matured CD103<sup>+</sup> DCs then migrate to mediastinal LNs and initiate Ag-specific immune responses via activation of naïve T cells. CD11b<sup>high</sup>CD103<sup>-</sup> cells are required for the induction of Ag-specific CD8<sup>+</sup> T cell priming (61). For example, during an influenza infection, only CD11b<sup>high</sup>CD103<sup>-</sup> cells can efficiently process and present Ags to MHC class I receptors in the lung. After migration, CD11b<sup>high</sup>CD103<sup>-</sup> cells then cross-prime CD8<sup>+</sup> T cells in the mediastinal LN. In the conducting airways, plasmacytoid DCs, defined by the expression of CD11c, B220, and plasmacytoid DC Ag-1, produce type I IFNs in response to viral infection and modulate regulatory T cells for lung homeostatic maintenance (62).

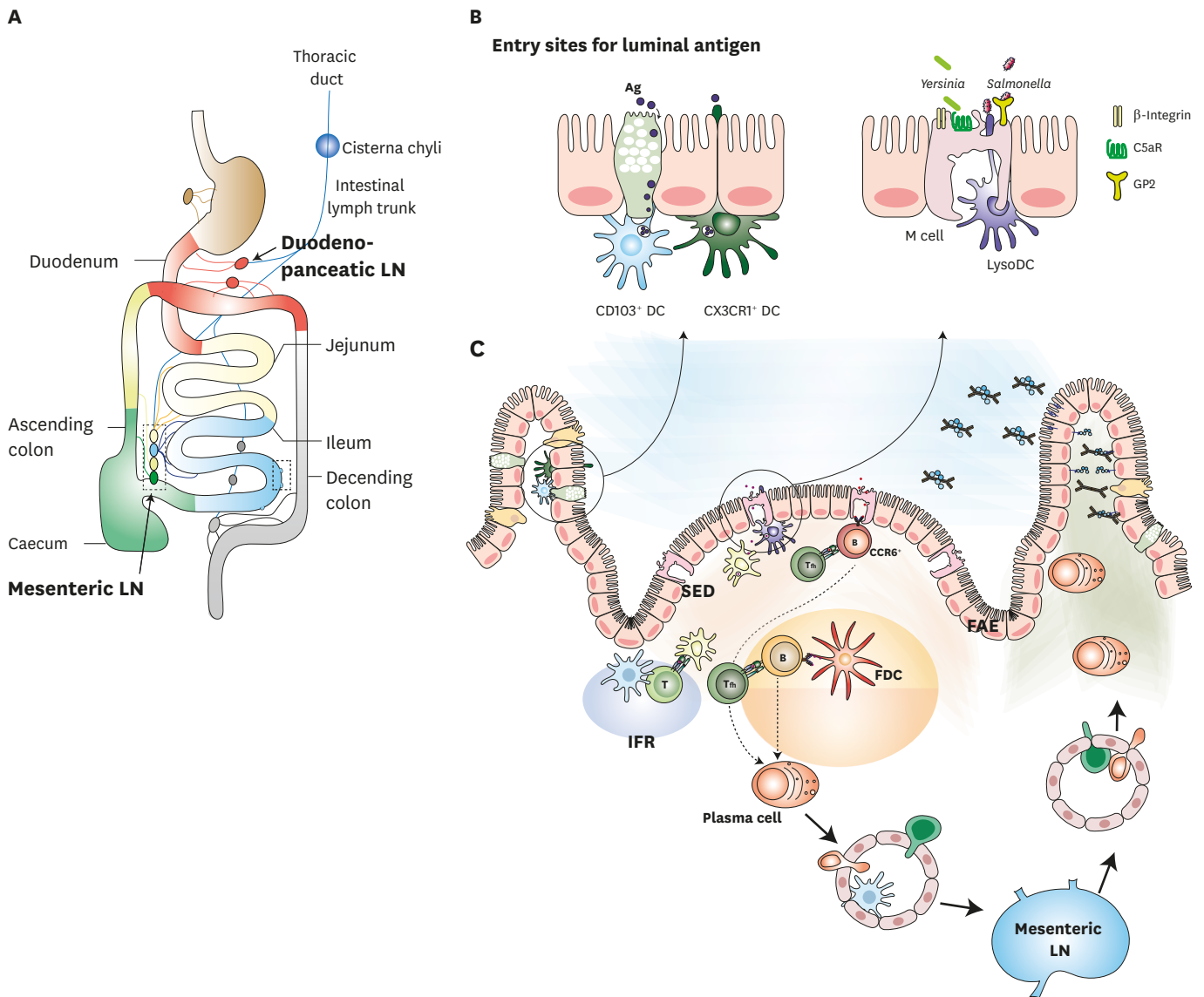
## INDUCTION AND REGULATION OF MUCOSAL ABS IN THE GASTROINTESTINAL SYSTEM

### Introduction of luminal Ags into the gastrointestinal immune system

The gastrointestinal immune system consists of GALT and LP. Since luminal Ag influx is tightly regulated by IECs, introduction of luminal Ags for priming Ag-specific adaptive immunity occurs primarily in M-cell-containing GALTs (27). Examples of GALTs include Peyer's patches (PPs), solitary isolated lymphoid tissues (SILTs), cecal patches, and colonic patches (1,22). These lymphoid tissues have different distributions across the gut segments; PPs are abundant in the terminal ileum but scarce in the duodenum. In the large intestine, cecal patches and colonic patches, which are lymphoid structures similar to PPs, are found in the appendix and in the colon and rectum, respectively (63). SILTs observed throughout the intestine contain cryptopatches, in which isolated lymphoid follicles mature (64). The distribution of mature SILTs is correlated with bacterial burden; in humans, isolated lymphoid follicles are concentrated in the ileum and rectosigmoid colon (65). Each regional lymph of the gastrointestinal mucosa, including regional GALT, drains along the length of the mucosa and flows through the thoracic duct into the blood circulation (22). For example, lymph from the duodenum of the small intestine and the transverse colon drains into duodenopancreatic LNs embedded in pancreatic tissue; lymph from the jejunum drains to the middle MLNs, and lymph from the ileum, caecum, and ascending colon collects in the distal segments of the MLNs (Fig. 3A). Given the differences in cellular components and environment along the length of the intestine, each draining LN conducts regional tissue-specific adaptive immune responses.

PPs are the best-characterized GALTs (66). PPs are separated from the lumen by FAE, which contains a limited number of goblet and Paneth cells and creates a thin mucus layer containing antimicrobial peptides. In the subepithelial dome (SED), MHC<sup>high</sup>CD11<sup>high</sup>CD11b<sup>+</sup>CD8a<sup>-</sup> cells inhibit the expression of Axin2-positive myogenic precursor by secreting IL-22 binding protein, which reduces the level of circulating IL-22 (67,68). This environment allows luminal Ags to access M-cell-containing FAE. In the M cells, the transport of luminal Ags is facilitated by transport receptors, such as GP2, C5aR, integrin





**Figure 3.** Induction of mucosal immune responses in the gastrointestinal tract. (A) Lymph from mucosal lymphatics collects for transport to the draining LNs. (B) Luminal Ags and pathogens, such as *Yersinia* and *Salmonella*, can be delivered to PP by M cells. This is facilitated by GP2, C5aR, and integrin  $\beta$ 1. Close to the M cells, Ags encounter lysozyme-expressing DCs. Luminal soluble Ags can be also transported into the LP via both the goblet cell-associated Ag passages of goblet cells and extending dendrites from CX3CR1<sup>+</sup> cells. (C) In PPs, the Ag-specific immune response was initiated in the GC, SED of PPs, and MLNs. Ag-specific effector cells then home to LP.

$\beta$ 1, and CD155 poliovirus receptor; these receptors are capable of binding whole luminal bacteria and soluble Ags (Fig. 3B) (27,31,69,70). For example, GP2 interacts with FimH of type I piliated bacteria, such as *Escherichia coli* and *S. typhimurium*, and can transport them into PPs (31). C5aR interacts with outer membrane protein H of *Y. enterocolitica* (69). Ags conjugated to M-cell-specific Ab (NKM 16-2-4), anti-GP2 Ab, or C5aR ligand are transcytosed via M cells, which can then induce Ag-specific immune responses (71,72). Lysozyme-expressing DCs that are localized to M cells extend their dendrites through M-cell-specific transcellular pores to the lumen, where they can take up luminal pathogens such as *S. typhimurium* (73).

In other LNs, Ags and soluble molecules flow from the afferent lymphatics into the LN parenchyma through a conduit network consisting of fibroblastic reticular cells and their

specialized collagen fibers (74). However, Ag influx into PPs mainly depends on M cells because PPs do not contain afferent lymphatics (27). Interestingly, a recent study reported a specialized conduit system in PPs (75). Intestinal fluid absorption occurs due to the local osmotic gradient via ion channels, after which the fluid flows from SED to the follicle and interfollicular regions (IFRs) of PPs. In this study, 14 kDa hen egg lysozyme labeled with Alexa Fluor 488 was shown to rapidly penetrate PPs and flow along the conduit network in explanted intestinal loops. Although the mechanistic link between conduit flow and immune induction remains unknown, disruption of the conduit flow has been linked to an impaired mucosal Ab response. Therefore, these observations suggest that regulation of Ag delivery within PPs is closely related to Ag-specific mucosal immune induction.

The LP underlying the intestinal villi is an immune effector site. Soluble Ags from the lumen can be delivered into the LP via goblet-cell-associated Ag passages and the extended dendrites of CX3CR1<sup>+</sup> cells (Fig. 3B) (76). In the LP, the Ags encounter CD103<sup>+</sup> DCs either directly or via connexin 43-expressing gap junctions. CD103<sup>+</sup> DCs that have taken up Ags migrate to MLNs in a CCR7-dependent manner to induce tolerance to the Ags (77).

### Induction of IgA responses in the gastrointestinal tract

Once Ags have translocated into PPs, they encounter the SED environment, which is a niche located between the FAE and B cell follicles. The SED contains double-negative cDCs, lysozyme-expressing DCs, macrophages, T cells, and B cells (78). Current evidence suggests that T-cell-dependent IgA induction is initiated following the cognate interaction between DCs and follicular helper T (Tfh) cells in the IFR of PPs (Fig. 3C). SED-localized DCs likely drive the initiation of Ag-specific immune responses, because their primary function is to capture translocated luminal bacteria, such as *E. coli*, SIgA-coated *Shigella flexneri*, and *S. typhimurium* (79). In addition, DCs bearing Ags or DCs stimulated by TLR7 ligands were reported to move from the SED into the IFR of PPs (80). Nevertheless, the mechanism by which Ags move from the SED to B cell follicles is poorly understood.

Recent studies showed that the early IgA response occurs without clonal selection in CCR6<sup>+</sup> B cells of the SED. This is in contrast to other LNs, in which B cell clones that exhibit low affinity to Ags fail to survive, and high-affinity Ag receptor-expressing B cell clones are preferentially selected for differentiation into early plasmablasts in GCs (81,82). CCR6<sup>+</sup> B cells localized to the SED generally encounter Ags that have been transcytosed by M cells. They then initiate CSR to IgA in response to cognate Ag stimulation without clonal selection; this process occurs after the expression of activation-induced cytidine deaminases in CCR6<sup>+</sup> B cells. Furthermore, low-affinity B cell clones extensively proliferate without any competition in a T-cell-dependent manner within the SED, even though some CD4<sup>+</sup> T cells in the SED are Tfh cells related to B cell clonal selection via T cell receptor-peptide-MHC class II interactions (83,84). However, low-affinity B cell clones fail to enter the pre-existing GCs; only high-affinity B cell clones are preferentially selected, after which they expand and undergo somatic hypermutation in pre-existing GCs of PPs.

PP GCs spontaneously arise during homeostatic response and continuously exist (85). In the GCs of peripheral LNs, follicular DCs (FDCs) present Ags as part of immune complexes to select for high-affinity B cells (86). It is currently unclear whether FDCs are necessary for the selection of high-affinity IgA-expressing B cell clones in PPs. FDCs are likely involved in the IgA response during GC reactions, as reports have shown that IgA deposition follows the distribution of the FDC network. In steady-state PPs, FDCs that have been stimulated

by TLR and retinoic acid receptor signaling express chemokines and survival factors, which facilitate IgA B cell differentiation (78,87). In addition, a cathelin-related antimicrobial peptide contributes to FDC stimulation, which in turn enhances IgA B cell differentiation (88). Following GC reactions, selected plasma precursor cells migrate through the lymph, which drains along the length of the mucosa into specific draining LNs. The plasma precursor cells then flow through the thoracic lymph duct into the blood circulation, after which they can home to the LP of the small and large intestines (8). This homing process is regulated by specific receptors. The microenvironment of PPs induces plasmablasts to express  $\alpha 4\beta 7$ , CCR9, and CCR10, which target the plasmablasts to the small and large intestine (89). In addition, mucosal memory cells and long-lived plasma cells were found in mice after infection and oral immunization. IgA<sup>+</sup> memory B cells can be characterized by their expression of IL-17 receptor C, IL-22 receptor subunit  $\alpha 2$ ,  $\alpha 4\beta 7$ , CCR9, and CCR10 (90). However, the generation and maintenance of IgA<sup>+</sup> memory B cells are not clearly understood. In addition to the T-dependent pathway, gut IgA can be generated by the T-independent pathway. The T-independent pathway is induced by the innate signaling of TLR ligands, a proliferation-inducing ligand, and B cell activation factor. A recent study reported that intestinal epithelial endoplasmic reticulum stress induces peritoneal B1b cells to differentiate into gut-homing IgA<sup>+</sup> plasma cells (91). These findings suggest that B cell responses induced by T-cell-dependent and -independent pathways may differ in their origin and activation signals.

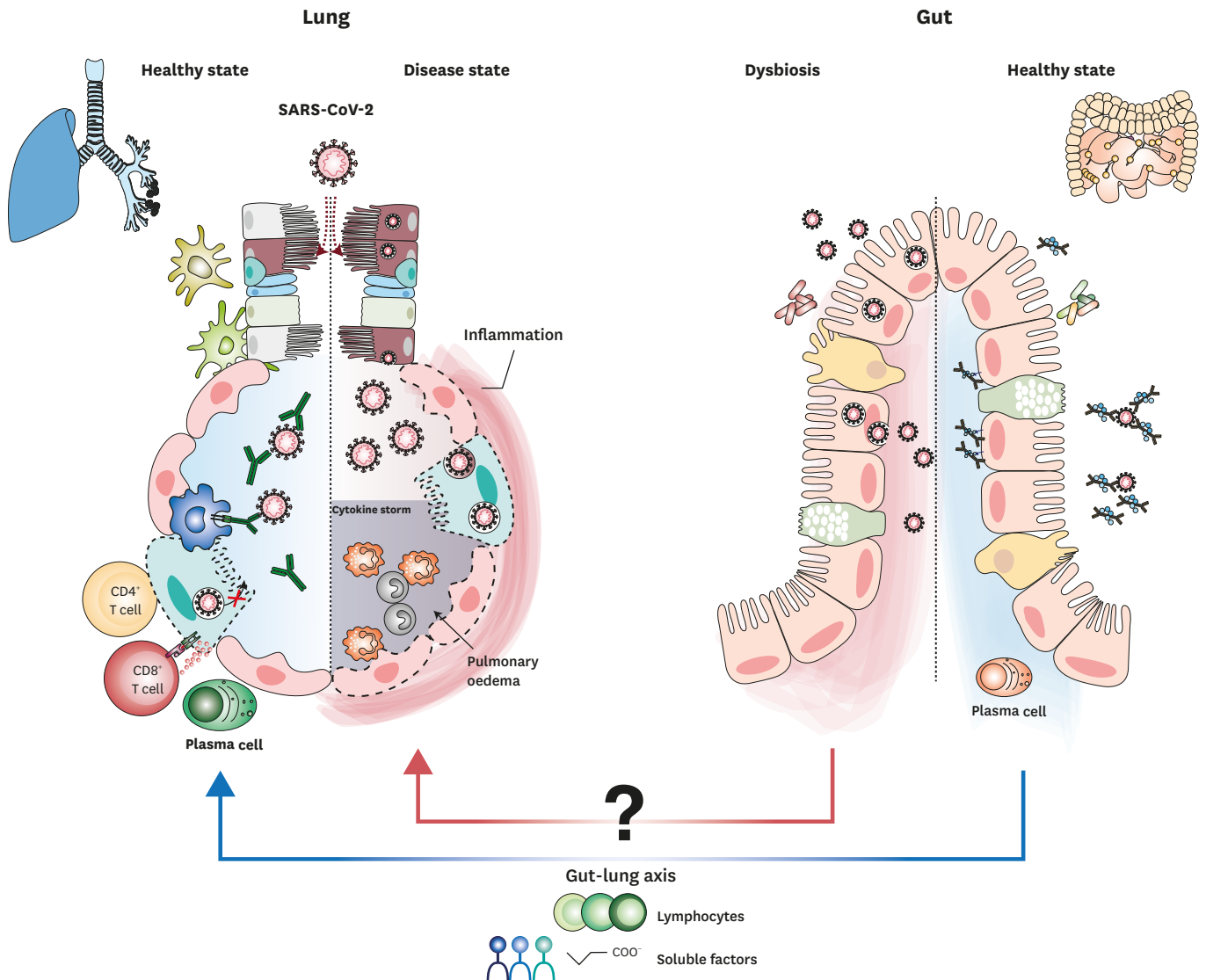
#### Other Ab responses in the gastrointestinal tract

Cooperation between SIgM and SIgA improves the prevention of bacterial dissemination (8). For example, the oral administration of *S. typhimurium* coated with SIgM and SIgA reduces mucosal infection and systemic dissemination of the pathogen. IgM-secreting plasma cells are observed mainly in the terminal ileum of humans, where they constitute 10%–20% of total plasma cells. In the gut, IgM<sup>+</sup>IgD<sup>-</sup>CD27<sup>+</sup> memory B cells can undergo IgA CSR in response to T-cell-dependent and -independent pathways; this strategy enables a rapid response to microbial infection. Additionally, commensal microbe-specific IgG is detected in intestinal mucosa and peripheral blood. The presence of the commensal bacterium *Akkermansia muciniphila* in mucus can activate bacteria-specific Tfh cells, resulting in the production of specific IgG1 in the gut (92). Furthermore, highly protective levels of IgG were identified in mice infected with *Citrobacter rodentium* (93). Pathogen-specific IgG, but not IgA or IgM, is necessary to eliminate pathogens from the intestinal mucosa, as IgG-coated pathogens are subsequently killed by neutrophils in the intestinal lumen.

## HOMEOSTATIC MAINTENANCE AND BREAKDOWN OF MUCOSAL FIREWALLS

Respiratory pathogen infections vary in their severity, from mild common colds to deadly pandemics. Recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic, known as coronavirus disease 2019 (COVID-19) (94). SARS-CoV-2 utilizes host receptors to enter into cells, namely human angiotensin-converting enzyme 2 (ACE2) and type II transmembrane serine protease (TMPRSS2). Several single-cell RNA sequencing reports suggest that ACE2<sup>+</sup>TMPRSS2<sup>+</sup> cells include ciliated and goblet cells in the nasal epithelium, AT2 cells in alveoli, and enterocytes in the ileum and colon (95,96). In addition, SARS-CoV-2 infection was detected in ciliated cells and AT2 cells in autopsied lungs using RNA in situ hybridization. Given that the role of AT2 cells is closely associated with alveolar regeneration, damage to AT2 cells by viral infection may lead to acute alveolar damage

(97). Although the immune response to SARS-CoV-2 has not yet been fully elucidated, it can be modelled using studies from other respiratory viruses, including SARS-CoV (Fig. 4). In the alveoli, SARS-CoV-2 infection in AT2 cells induces pyroptosis, which triggers the generation of pro-inflammatory cytokines from neighboring epithelial cells, endothelial cells, and AMs. The cytokines promote the recruitment of monocytes, macrophages, and T cells to the infected area, which then cause further inflammation. The attraction of virus-specific CD8<sup>+</sup> T cells facilitates the elimination of virus-infected cells, thus blocking further spread of the virus. Neutralizing Abs also inhibit viral infection and trigger AM phagocytosis of neutralized viral particles. These immune responses can clear the virus while causing only mild lung damage. Consequently, the level of adaptive immunity determines the severity of the disease. In contrast, hyperaccumulation of immune cells promotes excessive infiltration of mononuclear phagocytes, which can in turn induce a systemic cytokine storm, pulmonary edema, and pneumonia; the result is widespread inflammation and multi-organ damage.



**Figure 4.** Hypothetical immunity in the lung and gut during SARS-CoV-2 infection. SARS-CoV-2 infects both ACE2- and TMPRSS2-expressing epithelial cells, including ciliated cells, AT2 cells, and enterocytes. In mucosal compartments, induction of the adaptive immune response can promote viral clearance.

In addition to severe respiratory symptoms, some COVID-19 patients also develop gastrointestinal symptoms (94). Recent data suggest that SARS-CoV-2 targets ACE2<sup>+</sup>TMPRSS2<sup>+</sup> enterocytes, which also reside in the intestine; thus, the gut is likely to be a target organ for SARS-CoV-2 infection. SARS-CoV-2 has been shown to infect, replicate, and produce infectious viral particles in human epithelial cells and human small intestinal organoids (98). Given that ACE2 expression is decreased upon SARS-CoV binding, reduced ACE2 expression is also expected during SARS-CoV-2 infection. ACE2 dysfunction caused by decreased ACE2 expression may also affect the composition of the gut microbiota by suppressing the expression of neutral amino acid transporters in IECs, thereby decreasing nicotinamide levels (99). Therefore, ACE2 dysfunction during SARS-CoV-2 infection may be sufficient to alter the composition of the gut microbiota, as COVID-19 patients show a change in gut microbiota composition to increased opportunistic pathogens and often have decreased levels of butyrate-producing bacteria (99,100). While the impact of COVID-19 on gut disorders remains unclear, evidence suggests that COVID-19 influences the systemic dissemination of bacteria, endotoxins, and microbial metabolites. Additionally, it may trigger a systemic cytokine storm and multiorgan dysfunction. Therefore, we hypothesize that the effects of the gut microbiota on lung immunity may mediate the effects of COVID-19 on the gut-lung axis (Fig. 4). Additionally, we suggest that protective immune response against SARS-CoV-2 in the gut may be able to play an important role in controlling the pathogenesis of COVID-19.

## CONCLUDING REMARKS

In this review, we summarize the cellular components of the lung and gut. We also describe their crosstalk in Ag-specific immune responses, and the implications thereof for vaccine development in terms of the route of vaccine administration. Ag-specific immune responses in the mucosal compartment are particularly important for protecting against mucosal pathogens, including SARS-CoV-2, since Ab can block viral entry to the mucosal compartment. Although parenteral vaccination can induce protective IgG at the respiratory mucosa, mucosal immunization through the nasopharyngeal route can induce potent T cell and IgA responses in mucosal compartments, including the upper respiratory tract. Given that the currently available mucosal vaccines contain live-attenuated viruses, the development of a mucosal vaccine for COVID-19 may be challenging. Furthermore, weakly immunogenic vaccines, such as protein subunit vaccines, cannot be administered via the nasopharyngeal route due to the lack of safe adjuvants. Therefore, future studies are required to optimize currently available live-attenuated vaccines and mucosal adjuvants.

## ACKNOWLEDGEMENTS

This study was supported by grants from the Basic Science Research Programs (NRF-2019R1A2C2004711) through the National Research Foundation, funded by Korean Ministry of Science and ICT, and by the Basic Science Research Program through the NRF, funded by the Ministry of Education (2017R1A6A1A03015876). Dr. Yong-Suk Jang was supported by the Research Base Construction Fund Support Program funded by Jeonbuk National University in 2020.



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