Immunomodulation by endothelial cells — partnering up with the immune system?

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Abstract | Blood vessel endothelial cells (ECs) have long been known to modulate inflammation by regulating immune cell trafficking, activation status and function. However, whether the heterogeneous EC populations in various tissues and organs differ in their immunomodulatory capacity has received insufficient attention, certainly with regard to considering them for alternative immunotherapy. Recent single-cell studies have identified specific EC subtypes that express gene signatures indicative of phagocytosis or scavenging, antigen presentation and immune cell recruitment. Here we discuss emerging evidence suggesting a tissue-specific and vessel type-specific immunomodulatory role for distinct subtypes of ECs, here collectively referred to as ‘immunomodulatory ECs’ (IMECs).

We propose that IMECs have more important functions in immunity than previously recognized, and suggest that these might be considered as targets for new immunotherapeutic approaches.
node addressins, mostly in mucosal and lymphoid tissue) and chemokines (such as CCL2 and CXCL10) by ECs. During immune homeostasis, they allow patrolling immune cells to extravasate into tissue, and during inflammation, ECs can become activated and capable of actively recruiting effector immune cells\(^{11,12}\). EC activation can be induced by cytokines such as IL-6, IL-1β and tumour necrosis factor (TNF), but also by pathogen-associated molecular patterns, such as lipopolysaccharide\(^{19,20}\). The surface repertoire of adhesion molecules, selectins and addressins on ECs as well as their repertoire of secreted chemokines, in combination with the differential expression of cognate integrins, selectin ligands and chemokine receptors by immune cells, determines which circulating immune cells invade which tissue\(^1\). Some aspects of immune cell recruitment by ECs might differ between species (as is also the case for antigen presentation (see the next section and BOX 2)).

### Antigen presentation by ECs

Some EC subtypes are considered semi-professional APCs as they express genes involved in antigen capture, processing and presentation. For example, human renal vascular ECs express the major histocompatibility complex class II (MHC-II) surface molecule HLA-DR, which allows them to present antigens to CD4\(^+\) T cells\(^{21-23}\), and in vitro experiments showed that human umbilical vein ECs can activate allogenic T cells\(^{24-26}\). However, unlike professional APCs (such as dendritic cells), ECs generally do not express the surface receptors CD80 and CD86 (REF\(^{26}\)), which bind to CD28 on naive T cells and are required for their activation. ECs therefore primarily activate antigen-experienced T cells, although experiments in mice have shown that naive T cells can also be activated by ECs in the context of allostimmunity\(^{27,28}\). Importantly, not all molecules/processes related to APC function in ECs are conserved between species\(^29\) [<Box 2]. Interferon-γ (IFNγ) and TNF induce immunomodulatory processes in human and mouse ECs in vitro, including antigen uptake, processing and presentation\(^{30}\). Antigen presentation and immune cell recruitment by ECs contribute to allostimmunity and kidney/heart transplantation failure, for example through CD80 T cell-induced lysis of ECs in the donor tissue\(^{30-33}\). Moreover, antigen presentation by human ECs has been implicated in autoimmune diseases such as rheumatoid arthritis\(^34\).

There are estimated to be more than 10\(^{13}\) ECs in the human body\(^35\); thus, even if only a fraction of ECs acts as semi-professional APCs, they form a large reservoir of potential APCs. ECs contextually present intracellular and extracellular antigens depending on the EC subtype and activation status\(^36-38\). For the presentation of intracellular antigens by ECs, nicotinic oxide\(^39\) and IFNγ can induce a modified proteasome\(^40-42\), called the ‘immunoproteasome’, which facilitates antigen degradation and antigen loading\(^43\). ECs share many features with professional APCs, but differ from them in other aspects [Table 1]. For instance, ECs are exposed to shear stress\(^44\), which has been found to increase intercellular adhesion molecule 1 (ICAM1) expression\(^41-42\). ICAM1 binds to T cell integrins, which are capable of increasing T cell receptor signalling\(^45\). Moreover, shear stress increases the binding of selectins\(^45-48\), upregulates E-selectin expression in response to IL-1β\(^49\) and inhibits E-selectin expression in response to TNF\(^42\). Through binding to P-selectin glycoprotein ligand 1 on T cells, E-selectin can increase T cell receptor signalling, co-inhibitory molecule expression and T cell proliferation in the context of antigen presentation by ECs\(^50\). The roles of non-conventional MHC molecules such as MR1 (activating mucosal-associated invariant T cells\(^51\)) and BTN3A1 (presenting phosphoantigens to Vγ9Vδ2+ T cells\(^52\)) in antigen presentation in ECs have yet to be determined.

### Tissue-specific immunomodulation by ECs

Studies from the past two decades examined possible roles of ECs in immunomodulation at the bulk population level\(^53-55\). A recent transcriptomic and epigenomic study on bulk mouse ECs reported tissue-specific patterns of gene transcription, with notable differences in expression patterns of co-stimulatory molecules as well as chemokines and cytokines, suggesting tissue-specific immunomodulation by ECs\(^54\). Single-cell studies have now allowed deeper insights to be obtained into the role of EC immunomodulation in (1) the recruitment and homing of immune cells to lymph nodes, (2) the modulation of immunity in response to external challenges in the liver and lung, (3) the detection and clearance of immune complexes in the liver and kidney and (4) the shielding of the brain tissue parenchyma from immune cell invasion in healthy conditions.

### Lymphoid organs

Secondary lymphoid organs, such as lymph nodes and Peyer patches, and tertiary lymphoid organs that arise in response to chronic inflammation are of particular interest in the context of immunomodulation by ECs, as these form ‘hubs’ in the lymphatic system where cells of the innate and adaptive immune systems interact\(^56\). Lymph nodes contain a vascular bed with a heterogeneous composition of ECs that line arterioles, capillaries and venules. Notably, lymph nodes also contain

**Box 1 | Evolutionary origin of ECs**

From an evolutionary perspective, it is not surprising that certain subtypes of endothelial cells (ECs) have immunomodulatory properties. Indeed, in invertebrates, blood vessels initially consisted only of hollow matrix tubes, with motile haemocytes that patrolled the body for immune surveillance\(^57\) (see the figure, part a). Later in evolution as vertebrates developed, these haemoocytes diverged to become adherent ECs or immune cells, such as patrolling monocytes that scan the vascular lining for cellular debris\(^58\), neutrophils or tissue-resident immune cells such as macrophages\(^59\) (see the figure, part b). Given that tissue-resident immune cells such as macrophages also have tissue-specific characteristics\(^60-62\), one might speculate that immune cells and ECs have co-evolved to allow optimal tissue immune homeostasis.

**Box 2 | Evolution of ECs**

| a | Invertebrates |
|---|---|
| Haemocyte | BM, basement membrane. |

| b | Vertebrates |
|---|---|
| Patrolling monocyte | Extravasation |

BM, basement membrane.
high endothelial venules (HEVs); these are a subtype of postcapillary venules (PCVs) that are lined by high (tall and plump) ECs that are specialized in recruiting immune cells such as monocytes, plasmacytoid dendritic cell precursors, neutrophils, B cells and T cells^{12,73–79}. Naive T cells in the circulation home to lymph nodes, a process that, under non-inflamed conditions, is mediated by the adhesion molecule L-selectin, which binds to addressins on HEVs. These include adhesion molecules such as CD34, podocalyxin, GLYCAM1 or MADCAM1 containing the 6-sulfo sialyl Lewis X glycan modification. These modified adhesion molecules can be detected by antibodies binding peripheral node addressins, such as MECA-79 (REFS^{58–59}). A combination of addressins and chemokines such as CCL21 facilitates the capture and tethering of naive T cells on HEVs and promotes their extravasation^{57} (FIG. 1a). HEVs are extensively remodelled upon infection and the subsequent expansion of draining lymph nodes^{66}, but their phenotypic plasticity is only beginning to be explored.

An outstanding question is whether the interaction between HEVs and immune cells is sufficiently long to allow immunomodulation by the ECs. For T cells, which can reside in lymph node ‘pockets’ close to HEVs^{57}, the interactions may be long enough to allow HEVs to modulate T cell activity and differentiation through the expression of co-inhibitory or co-stimulatory receptors and the secretion of cytokines. However, this might be a T cell/HEV-specific phenomenon, given that transendothelial migration of immune cells across conventional PCVs, which are the primary site of immune cell recruitment in many organs, is rapid^{11–14} (for example, 6 min for mouse neutrophils in vivo^{16}), which limits sustained interactions with ECs. In the liver, lungs and kidneys, however, immune cell recruitment occurs primarily in capillaries, which are often only a few micrometres in diameter^{30,31}. This causes immune cells to crawl, slows down extravasation and prolongs interactions with ECs, potentially allowing immunomodulation by ECs.

The characterization of HEVs at single-cell resolution under inflammatory conditions has strengthened the concept that HEVs can modulate immune cells (FIG. 1b). Indeed, scRNA-seq analysis of enriched mouse MECA-79^{+} HEVs from lymph nodes, isolated after oxazolone-induced inflammation (which promotes HEV activation^{68}), revealed an upregulation of EC activation markers and the co-stimulatory molecule CD137, which can suppress the activation of immune cells that express CD137L^{69} such as dendritic cells^{70}. Activated HEVs from oxazolone-exposed mice also express higher levels of macrophage migration inhibitory factor (MIF), which regulates context-dependent M1/M2 macrophage polarization^{71}, and thrombospondin 1 (TSP1), which can impair T cell activation^{72}. Together, these findings suggest that HEVs have immunomodulatory functions beyond immune cell recruitment^{73}. Another scRNA-seq study of mouse lymph nodes implied that non-HEV ECs can recruit myeloid cells to lymph nodes during inflammation in a MECA-79-independent, but P-selectin and E-selectin-dependent manner^{74}, implying that not only HEVs are important for (myeloid) immune cell recruitment during inflammation (FIG. 1b). Single-cell studies in mouse and human tumours further revealed that there is no clear phenotypic separation between HEVs and (postcapillary) venous ECs in tumours, which express a selected set of canonical and non-canonical HEV markers^{75,77}.

Interestingly, a combination therapy consisting of anti-VEGF therapy (which facilitates vessel normalization) and anti-PD-L1 immunotherapy promotes HEV formation and T cell recruitment, and improves antitumour immunity in preclinical tumour models^{75,76}. Similarly, the treatment of mice with anti-PD-1 in combination with delivery of vascular-targeted LIGHT proteins that induce non-canonical NF-κB signalling, which is required for differentiation of ECs into the HEV phenotype, induces HEV biogenesis and improves tumour immunity and immunotherapy in preclinical tumour models^{77,78} (FIG. 1c). Thus, in addition to the established function of HEVs in immune cell trafficking to lymph nodes during infections, HEVs may also have direct immunomodulatory effects. Further insight into this additional immunomodulatory potential and the extralymphatic biogenesis of HEVs during (chronic) inflammation, cancer and other diseases may offer new immunotherapeutic opportunities for these conditions.

**Organs controlling immunity versus tolerance to external danger**

Several organs, such as the liver, intestines, lung and skin, are exposed to airborne or nutrient-derived antigens, pathogens and toxins and to their microbiota, as well as microbiota-derived antigens (FIG. 1e). These organs must both protect the organism against harmful attacks by raising an adequate immune response and, at the same time, prevent uncontrolled or excessive immune attacks against harmless agents by inducing tolerance — a delicate balance that requires fine-tuned immunoregulation.

**The liver.** The liver is exposed to microbial and dietary antigens from the gut via the portal vein. Specialized EC subpopulations in the liver contribute to immune tolerance, most notably LSECs. LSECs are equipped with a repertoire of molecules for the detection and uptake of extracellular antigens (microbial products and viruses), including Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR6, TLR8, TLR9 (REFS^{81,82}) and scavenger receptors such as the C-type lectin receptor mannose receptor. In mice, LSECs take up and cross-present extracellular antigens on MHC-I molecules to CD8^{+} T cells, but have a tolerogenic function because they express high levels of co-inhibitory molecules such as PD-L1
and do not express (or express at only low levels) the co-stimulatory receptors CD80 and CD86, which are necessary for the activation of naive T cells\(^85\)–\(^87\). Similarly, exogenous antigens, acquired through mannose receptor-mediated endocytosis and presented on MHC-II molecules to naive CD4\(^+\) T cells, induce tolerance by promoting differentiation of regulatory T cells (T\(_{reg}\)) cells\(^96\)–\(^99\) [Fig. 1e]. Additionally, LSECs are also involved in Fc receptor-mediated phagocytosis and degradation of (primarily large) antibody–antigen immune complexes from the circulation\(^100\) [Fig. 1f].

LSECs recruit different immune cells via different molecular mechanisms. For example, T\(_{reg}\) cells migrate through the liver sinusoidal endothelium primarily by interacting with the scavenger receptor stabilin 1 and the adhesion molecules ICAM1 and VAP1, whereas CD8\(^+\) T cell extravasation into the liver is mediated primarily by ICAM1 [REFS\(^1\)–\(^3\)]. As LSECs exhibit zone-dependent heterogeneity in liver lobules\(^4\)–\(^6\), these findings raise the question of whether LSEC heterogeneity might contribute to zone-specific recruitment of T\(_{reg}\) cells and accompanying immunosuppression in the liver. A recent study showed that resident myeloid and lymphoid cells cluster around periportal regions of the liver, which tunes TCR signalling\(^d\) [Fig. 1g]. The resulting perportal concentration of immune cells was more efficient than a uniform distribution of immune cells in protecting against systemic bacterial dissemination. This demonstrates that LSECs actively orchestrate the localization of immune cells, which optimizes host defence.

However, single-cell studies revealed confounding results. Indeed, the transcriptome of periportal LSECs differs from that of central vein LSECs in the human liver. Central vein LSECs upregulate the expression of CD32B (also known as FGGR2B; encoding an inhibitory receptor) and STAB1 (encoding stabilin 1) and of genes involved in innate immunity, phagocytosis and leukocyte activation, whereas periportal LSECs exhibit a TNF activation signature and express other immunomodulatory genes\(^8\). However, a paired-cell RNA-seq study of human liver LSECs revealed a unique gene expression program that is distinct from that of human hepatic sinusoidal endothelial cells\(^9\) [Fig. 1g].

| Category | Specific feature | APC | Described in ECs |Refs |
|--------|-----------------|-----|------------------|-----|
| **Immunological synapse** | | | | |
| **Antigen presentation** | | | | |
| MHC-I | Yes | Yes | \(28\) |
| MHC-II | Yes | Yes | \(29\) |
| CD1 (glycolipid antigens) | Yes, context dependent\(^a\) | | \(35,105,106\) |
| MR1 (metabolite antigens) | Unknown/not examined | NA | | |
| BTN3A1 (phosphoantigens) | Unknown/not examined | NA | | |
| **Co-stimulation** | | | | |
| CD80, CD86, CD58, CD275, CD252, CD137L, CD154, CD70 | Yes | Yes | \(28,167\) |
| **Co-inhibition** | | | | |
| PD1L, PD2L, CD155 | Yes | Yes | \(29\) |
| **Cytokines** | | | | |
| IL-1, IL-3, IL-5, IL-6, IL-8, IL-10, IL-11, G-CSF, GM-CSF, MCP1, M-CSF, CCL5, TGF\(\beta\), TNF | Yes\(^c\) | Yes, polarization of immune cells by ECs during activation | \(35\) |
| **Receptor/signalling pathways** | | | | |
| **Extracellular/ intracellular** | | | | |
| TLR1–TLR4, TLR6, TLR8, TLR9 | Yes | Yes | \(188\) |
| **Intracellular** | | | | |
| p38–JNK–STAT–JNK signalling | Yes | Yes, relevance of p38 signalling | \(29,309,370\) |
| **Antigen uptake/processing** | | | | |
| Uptake | Phagocytosis | Yes | Yes, population dependent | \(13,148,171\) |
| Processing | Immunoproteasome | Yes | Yes, context dependent | \(37,38\) |
| **Extracellular factors** | | | | |
| Mechanical | Shear stress | No | Affects selectin and CAM expression and function, possibly tunes TCR signalling\(^d\) | \(42,45,46,48,119\) |
| **Cell–cell interaction** | | | | |
| Interaction duration | Long (several hours) | Population dependent?\(^d\) | \(63,64\) |

Although some of the features indicated in the table remain speculative and require further investigation, antigen-presenting cell (APC) characteristics in professional APCs and endothelial cells (ECs) can overlap and differ in several manners. BTN3A1, butyrophilin subfamily 1 member A1; CAM, cell adhesion molecule; CD, cluster of differentiation; DC, dendritic cell; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; JAK, Janus kinase; JNK, JUN amino-terminal kinase; M-CSF, macrophage colony-stimulating factor; MCP1, monocyte chemoattractant protein 1; MHC, major histocompatibility complex; MR1, major histocompatibility complex class I-related gene protein; NA, not applicable; PD1L, programmed death ligand 1; STAT, signal transducer and activator of transcription; TCR, T cell receptor; TGF\(\beta\), transforming growth factor-B; TLR, Toll-like receptor; TNF, tumour necrosis factor. \(^a\)Not shown in human ECs. \(^b\)CD80/CD86 not ubiquitously expressed by ECs, only in vitro in human ECs. \(^d\)Polarization towards pro-inflammatory/anti-inflammatory cytokine secretion. \(^d\)These characteristics are speculative.

Table 1 | Comparison of APC features in professional APCs (DCs and macrophages) and ECs

LSECs also affect disease outcome. For example, LSECs present cancer cell-derived apoptotic bodies to naive CD8\(^+\) T cells. However, as LSECs act as semi-professional APCs, they impair the differentiation of naive CD8\(^+\) T cells into cytotoxic effector T cells, which are capable of killing cancer cells, thereby hampering tumour immunity. It was shown that breaking LSEC-induced
Immune cell homing into lymph nodes through HEVs

a. Naive T cell recruitment

- Tn cell
- Chemokine
- Addressin
- HEV
- Extravasation

b. Immune cell recruitment by HEVs and venous* ECs during inflammation

- Monocyte
- Effector T cell
- Neutrophil
- Selectin
- HEV
- Venin


c. Role of HEVs in cancer immunotherapy

- AAT or LIGHT, plus ICB, promotes tumour HEV induction*
- Improved tumour immunity and immunotherapy*


d. Additional immunomodulation by HEVs* (?)

- Inverse signalling through CD137L?
- MIF-induced macrophage polarization?
- Inhibition of T cell activation by TSP1?

- HEV
- MIF
- M1/M2-like differentiation

- HEV
- TSP1
- T cell
- Activation

Immune tolerance by liver IMECs

e. Tolerance to foreign (gut flora-derived) antigens

- CD8+ T cell
- CD4+ T cell
- Gut flora
- PDL1
- Suboptimal activation
- LSEC

- Treg cell induction
- Treg cell
- T cell
- Gut flora
- MHC class I
- TCR
- MHC class II

- Uptake
- Immune complex

f. Clearance of immune complexes from the circulation by LSECs

- PLVAP+ TEC
- MIF-induced macrophage polarization?
- Improved tumour immunity and immunotherapy*

- T cell
- Activation

- HEV
- TSP1
- T cell
- Activation

- HEV
- MIF
- M1/M2-like differentiation

- HEV
- TSP1
- T cell
- Activation

- LSEC
- Tumour hepatocyte
- Treg cell
- Antimetastatic therapy
- FOLR2
- Treg cell
- M1/M2-like differentiation

- LSEC
- FOLR2
- Macrophage

- T cell
- Treg cell
- Metastasis

- FOLR2
- Macrophage
- T cell
- Treg cell
- Metastasis

- FOLR2
- Macrophage
- T cell
- Treg cell
- Metastasis

i. Macrophage modulation in fibrosis?*

- In cirrhosis, ACKR1+ ECs in a fibrotic niche can recruit and might induce context-dependent macrophage polarization (e.g. by GAS6)?

- Disease-specific macrophage

- Endothelial cell
- ACKR1

- GAS6

- Hepatic cell

- LSEC

- T cell
- Treg cell
- Metastasis

- T cell
- Treg cell
- Metastasis

- T cell
- Treg cell
- Metastasis

- T cell
- Treg cell
- Metastasis

- T cell
- Treg cell
- Metastasis
immune tolerance (using nanoparticles to deliver melittin, a host defence peptide with immunomodulatory activity) leads to LSEC activation and a changed hepatic chemokine and cytokine milieu, which inhibits metastasis in melanoma, breast cancer and colon cancer models. In mouse models of hepatocellular carcinoma, malignant hepatocyte-derived vascular endothelial growth factor (VEGF) induces plasmalemma vesicle formation to increase HEV biogenesis, thereby promoting tumour immunity and immunotherapy. Interestingly, activated HEVs express additional immunomodulatory genes, which may impair dendritic cell (DC) activation (via reverse CD137–CD137L signalling), alter macrophage differentiation (via macrophage migration inhibitory factor (MIF)) or inhibit T cell activation (via thrombospondin 1 (TSP1)). Liver ECs with immunomodulatory properties (these are mostly liver sinusoidal ECs (LSECs)) facilitate tolerance to harmless gut flora-derived antigens through co-inhibition of CD8+ T cells via the checkpoint ligand programmed death ligand 1 (PD-L1) upon cross-presentation of gut flora-derived antigens via major histocompatibility complex (MHC) class I through the induction of regulatory T cells (Treg cells) upon presentation of gut flora-derived antigens to CD4+ T cells by MHC class II.

Liver ECs with immunomodulatory properties (these are mostly liver sinusoidal ECs (LSECs)) form a communication hub in the liver. Known and putative insights into immunomodulation by ECs in lymph nodes and liver.

**Immunomodulation by ECs in lymph nodes and liver.** Known and putative insights into immunomodulation by endothelial cells (ECs) in lymph nodes and the liver. a | Lymph nodes contain high endothelial venules (HEVs), which express chemokines, adhesion molecules and other surface molecules (addressins) that facilitate the adhesion or recruitment of lymphocytes such as naïve T cells (T0 cells), b | During inflammation (indicated by the red background), HEVs (upper panel) and venous ECs (bottom panel) in lymph nodes can recruit various immune cells, such as neutrophils, monocytes and effector T cells, in a selectin-dependent manner. c | In preclinical models of cancer, including breast cancer, melanoma that has metastasized to the lung and pancreatic cancer, anti-angiogenic therapy (AAT) or delivery of LIGHT protein, combined with immune checkpoint blockade (ICB), was found to increase HEV biogenesis, thereby promoting tumour immunity and immunotherapy. d | Interestingly, activated HEVs express additional immunomodulatory genes, which may impair dendritic cell (DC) activation (via reverse CD137–CD137L signalling), alter macrophage differentiation (via macrophage migration inhibitory factor (MIF)) or inhibit T cell activation (via thrombospondin 1 (TSP1)). e | Liver ECs with immunomodulatory properties (these are mostly liver sinusoidal ECs (LSECs)) facilitate tolerance to harmless gut flora-derived antigens through co-inhibition of CD8+ T cells via the checkpoint ligand programmed death ligand 1 (PD-L1) upon cross-presentation of gut flora-derived antigens via major histocompatibility complex (MHC) class I or through the induction of regulatory T cells (Treg cells) upon presentation of gut flora-derived antigens to CD4+ T cells by MHC class II. f | LSECs clear immune complexes from the circulation via uptake and degradation. g | Perportal LSECs sense gut bacteria and recruit resident macrophages and lymphocytes through chemokine gradients. Besides zone-specific immunomodulation, LSECs might form a hub for communication with resident macrophages through cytokine signalling, thereby altering macrophage phenotypes in a context-dependent manner. h | In hepatocellular carcinoma, malignant hepatocyte-derived vascular endothelial growth factor (VEGF) induces plasmalemma vesicle-associated protein-positive (PLVP7) tumour ECs (TECs) to form an immunosuppressive niche of folate receptor-β-positive (FOLR2+) macrophages and Treg cells. Therapeutic approaches that break LSEC-mediated immune tolerance can impair liver metastasis in preclinical models of metastatic melanoma, breast carcinoma and colon carcinoma. i | In regions of liver fibrosis, atypical chemokine receptor 1-positive (ACKR1+) ECs might recruit and modulate/polarize macrophages through the secretion of differentiation factors such as the protein GAS6, growth arrest-specific protein 6 (GAS6) in a contextual manner. Asterisks indicate recent insights which we considered novel for immunomodulatory EC biology. TCR, T cell receptor.

**The lung.** The lung is highly vascularized with a specialized composition of ECs, consisting largely of microvascular ECs that facilitate gas exchange between the circulation on the apical side and the air in alveoli on the basal side. Inhalation of large volumes of air exposes the lung to pathogens and pollutants, to which appropriate immune responses are required that do not put the vital gas exchange apparatus at risk. The lung has elaborate mechanisms to ensure homeostasis and dampen immune activation following lung damage. Immunomodulation by ECs might play a more important role in the lung than originally anticipated.

Indeed, compared with mouse ECs from the heart or brain, the gene expression signature as detected by bulk RNA-seq of lung ECs showed a marked upregulation of transcripts involved in immune regulation. Moreover, subsets of lung ECs express MHC-II, and in humans this feature appears to be restricted to capillary ECs. A recent scRNA-seq study revealed that human bronchial ECs form a transcriptomically distinct population from alveolar ECs, although the genes involved in immunomodulation do not appear to be their most distinguishing feature. Another single-cell study suggested that human alveolar capillary ECs can be divided in two populations on the basis of their transcriptome and location, where ECs termed ‘aerocytes’ (which are located close to alveolar type 1 epithelial cells) are specialized in gas exchange and immune cell recruitment, whereas general capillary ECs can activate CD4+ T cells through MHC-II, suggesting that these alveolar ECs might facilitate an adequate immune response against harmful antigens.

Though yet to be confirmed, VEGF may contribute to preventing uncontrolled, detrimental immune responses to the (commensal) microbiota. Indeed, a single-cell analysis of alveolar cell populations (conserved in humans, mice, rats and pigs) predicted capillary ECs to be the cell type most responsive to VEGF.
(released primarily by alveolar type 1 cells and secretory epithelial cells\(^1\)). Given the immunosuppressive effects of VEGF\(^1\), the aforementioned finding raises the question of whether VEGF signalling in the alveolar microenvironment might contribute to EC-mediated tolerance to airborne pathogens and toxins in the lung. Whether additional molecular mechanisms contribute to the tolerogenic nature of lung ECs with immunomodulatory features requires further study.

Emerging evidence also indicates that immunomodulation by pulmonary ECs may co-determine disease severity and

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**Perspectives**

**Dual role of lung IMECs in immunity versus tolerance**

| a Lung cancer | ↑ Tolerance |
|-------------|------------|
| TECs impair immune cell recruitment by downregulation of HLA, ICAM1, chemokines |
| TECs express inhibitory molecules such as PDL1 |
| Apoptosis |
| ↓ ICAM1 |
| ↓ HLA |
| ↓ Chemokine |
| Immune cell |
| CD8+ T cell |
| FAS |
| FASL |

| b Malaria infection | ↑ Immunity |
|----------------------|------------|
| Chronic inflammation induces HEV expansion |
| EC cytolysis |
| CD8+ T cell |
| TCR |
| Parasite |
| Parasite antigen |
| Vascular leakage and lung damage* |

| c Antigen presentation | ↑ Tolerance |
|------------------------|------------|
| Lung IMECs present foreign antigens to CD4+ T cells, which causes tolerance via induction of T<sub>reg</sub> cells — VEGF may promote this process* |
| CD4+ T cell |
| Parasite |
| Antigen |
| MHC class I |
| MHC class II |
| Pathogen |
| T<sub>reg</sub> cell |

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**Kidney ECs**

| d Kidney disease | Known |
|------------------|-------|
| **Health** |
| Thick glycocalyx |
| Glomerular EC |
| Impaired immune cell recruitment |
| **Disease** |
| Loss of glycocalyx |
| CAM |
| Selectin |
| Immune cell infiltration |

| e Immune complex detection and clearance* | Known |
|-----------------------------------------|-------|
| Immune complex |
| Glomerular EC |
| DSA target |
| Degradation |
| Macrophage |

| f Alloimmunity | Known |
|----------------|-------|
| DSAs activate ECs, possibly affecting context-dependent immunomodulatory properties |
| Glomerular EC |
| Donor kidney EC |

| g Compartment specificity* | Known |
|----------------------------|-------|
| Osmolar heterogeneity in different kidney compartments and glycocalyx thickness might affect EC inflammatory status |
| Glomerular EC |
| Medullary EC |

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**Immune privilege maintained by brain IMECs**

| h BBB | Known |
|-------|-------|
| Low immunomodulation by healthy BBB ECs prevents immune cell infiltration |
| TEC |
| CAM |
| Selectin |

| i Ageing* | Known |
|-----------|-------|
| Age-associated heterogeneity in proinflammatory gene signalling and VCAM1 expression in different EC subtypes, possibly increasing immunomodulation and immune cell recruitment and breaking the immune privilege of the brain |
| EC subtype |
| Inflammatory signalling |
| ↑ IFN |
| ↑ TLR |
| ↑ TGF |

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progression in lung cancer. Tumour ECs (TECs) from individuals with untreated, non-metastatic non-small-cell lung cancer of the squamous cell or adenocarcinoma subtype exhibit decreased expression of genes encoding ICAM1, the chemokines CCL2 and CCL18, the cytokine IL-6 and HLA-I/HLA-II (REF. 14), suggesting an immunosuppressive environment. Additionally, TECs of human and mouse lungs show elevated expression of genes encoding FASL, a cell death regulator capable of inducing cell death in cytotoxic T cells14, and of co-inhibitory molecules such as PD-L1, further indicating an immunosuppressive role (FIG. 2a).

Another single-cell study, of human and mouse lung tumours, illustrated a complex immunomodulatory gene signature5. In line with earlier studies, lung capillary TECs expressed lower levels of immunomodulatory genes (involved in antigen presentation and processing) than peritumoural capillary ECs, suggesting that certain TEC subpopulations might become more tolerogenic5. However, tumours had fewer capillaries, which suggests that further research is required to investigate the exact immunomodulatory role of lung capillary TECs8. Furthermore, mice with a deficiency of MHC-II in non-haematopoietic cells had fewer T<sub>reg</sub> cells in the lung and a lower pulmonary metastasis burden in lung tumour models14, which may suggest that antigen presentation by pulmonary ECs contributes to immune tolerance in lung cancer, although EC-selective knockout approaches are required to confirm this. However, another population of activated PCV lung ECs that was enriched in human non-small-cell lung cancer and mouse lung tumours was shown to upregulate a HEV-like gene signature and ACKR1 expression, suggesting that there may be different populations of TECs that either promote or suppress tumour immunity7. Notably, mass cytometry revealed high surface expression of HLA-DRA on healthy capillary lung ECs, which was comparable to that on immune cells in general. This finding requires further functional validation, but highlights the immunomodulatory potential of these ECs as non-professional APCs.

The role of lung ECs has also been investigated in various infection models. For example, in a mouse model of Plasmodium berghei-induced malaria, lung ECs were shown to cross-present malaria parasite antigens to CD8<sup>+</sup> T cells (this was also shown in vitro) in response to stimulation by IFNγ, which is presumably secreted by CD8<sup>+</sup> T cells (and possibly CD4<sup>+</sup> T cells and natural killer cells). This process is associated with vascular leakage and lung damage17 (FIG. 2b), indicating that antigen presentation by lung ECs can have detrimental effects. Vascularized lung-on-chip models allow investigation of the role of lung ECs in infections such as COVID-19. These showed that lung ECs underlying epithelial cells can be directly infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and contained viral RNA (however, without signs of active viral replication), and infected ECs exhibited a decreased barrier integrity30. In aged mice, pulmonary capillary ECs have been shown to upregulate various cytokine transcripts (such as Il1b, Tnf and Tgfβ1)31, which suggests that capillaries might contribute to lung diseases that are more prevalent in individuals >65 years of age, such as chronic obstructive pulmonary disease and lung cancer32, and possibly might contribute to the severity of COVID-19 (REF. 14). Given that aged individuals are more prone to severe COVID-19, it is possible that SARS-CoV-2 infection of ECs in aged individuals might lead to a more pronounced loss of barrier function and increased hyperinflammation in the lung33. On the other hand, SARS-CoV-2 infection of ECs in a human lung-on-a-chip model has also been shown to decrease CD31 expression and thus impair immune cell recruitment to the lung34.
The kidney
Kidney ECs represent a particularly heterogeneous population, where cortical, glomerular and medullary ECs exert distinct functions in the renal vascular bed and are exposed to different microenvironments depending on where they are located alongside the nephron125,126. Glomerular and peritubular ECs have fenestrations and are exposed to different concentrations of uremic toxins, which are filtered from blood, and different osmolalities, which may affect their phenotype and their responses to vasoregulation by the renin–angiotensin–aldosterone system125. Indeed, in vitro, elevated sodium chloride concentrations increase the expression of VCAM1 and E-selectin in human ECs and promote the transmigration of mononuclear immune cells and monocytes, and in vivo, higher salt concentrations enhance myeloid cell binding to ECs125,126. In agreement with these observations, newly identified subpopulations of cortical and medullary capillary ECs in healthy kidneys of mice express an interferon-regulated gene expression signature, including an upregulation of MHC-II, the functional consequences of which need to be validated127,128 (fig. 2g). Interestingly, medullary capillary ECs from dehydrated mice, which are exposed to non-physiologically high osmolalities, lower their transcriptional response to IFNβ125, indicating that different osmolalities may influence inflammatory responses via their effects on kidney ECs.

To date, studies of the immunomodulatory potential of ECs in the kidney have focused mainly on glomerular ECs. Glomeruli are the blood-filtering hubs of the nephron and contain fenestrations, which allows them to be selectively permeable to water, salts and specific macromolecules. Compared with other ECs, glomerular ECs have a particularly thick filamentous glyocalyx that contributes to the regulation of fluid balance, but also prevents interactions with immune cells. Upon activation of glomerular ECs in response to infection or as a consequence of disease, such as lupus nephritis, shedding of the glyocalyx exposes surface molecules on ECs that facilitate the extravasation of immune cells into the glomeruli125,130,131 (fig. 2d). This can contribute to immune cell-mediated damage of glomeruli when immune cells such as neutrophils infiltrate the glomeruli and release their granules125. Glomerular ECs also participate in immune responses by filtering circulating immune complexes from the blood into the glomeruli via transcellular transport, where these are removed by glomerular macrophages, which can also initiate an inflammatory response if appropriately stimulated129 (fig. 2e).

Immunomodulation by renal ECs is of particular interest in the context of organ transplantation. Renal microvascular ECs are frequently targets of donor-specific antibodies that bind to HLA molecules expressed by the transplanted kidney, and ECs contribute to alloimmunity by upregulating HLA-II genes after transplantation125,125 (fig. 2f). A recent study of transplanted human kidneys documented a not further specified subpopulation of donor ECs in the transplanted kidney that showed signs of activation125 (suggesting that it is a target of donor-specific antibody-mediated rejection) and an upregulation of genes involved in phagocytosis125, which may indicate antibody uptake. Also, under stress conditions, renal ECs (subtype to be specified) produce transforming growth factor-β (TGFβ)135 and can secrete large amounts of IL-6 (ref. 136). These cytokines can promote the differentiation of naive CD4+ T cells into either immunosuppressive Treg cells (when only TGFβ is present) or pro-inflammatory T helper 17 (Th17) cells (when TGFβ and IL-6 are present)137. As antigens presented by MHC-II molecules on renal ECs can skew CD4+ T cell differentiation towards either Treg cells or Th17 cells138–140, the inflammatory context on renal ECs are exposed to might have an impact on kidney transplantation success.

Thus, different renal EC populations appear to exert distinct immunomodulatory functions during homeostasis and inflammation and require further study. Therapeutic strategies targeted at ECs in donor kidneys before transplantation may allow the tweaking of EC-mediated immunomodulation in such a way that alloimmunity is decreased and transplantation success increased. Finally, in Wilms tumours, a cancer affecting the kidneys, renal TECs upregulate ACKR1 transcription141. Whether the potential for immune cell recruitment by ACKR1+ TECs can be exploited by tuning additional TEC populations to acquire ACKR1 expression to stimulate tumoricidal immune cell infiltration might be of interest as antitumor therapy, given the generally immunosuppressive features of TECs.

The brain
In healthy conditions, the brain is poorly infiltrated by immune cells owing to the low expression of adhesion molecules by the specialized capillary and PCV ECs of the blood–brain barrier (BBB)142 and the abundance of tight junctions between these ECs. Brain ECs thus exhibit a larger level of immune anergy and contribute to the maintenance of the immune privileged state of the brain143. Unlike liver and renal ECs, BBB ECs lack fenestrations and form continuous intercellular junctional complexes, limiting paracellular leakage of molecules from the circulation into the brain. Further, BBB ECs not only express low levels of adhesion molecules (such as ICAM1) but also express lower levels of cytokines and chemokines (such as IL-8 and CCL2), regulated in part by astrocyte-derived sonic hedgehog, which, via hedgehog receptors, induces immune quiescence in ECs, impairing immune cell migration144.

However, in models of infection or inflammatory disease, BBB ECs upregulate adhesion molecules (such as E-selectin and P-selectin) and chemokines (such as CXCL11), thereby promoting immune cell infiltration and inflammation in the brain145,146 (fig. 2f). For example, after transmigration, extravasated monocytes differentiate into Treg17-polarizing dendritic cells in response to brain EC-derived granulocyte–macrophage colony-stimulating factor (GM-CSF) and TGFβ147, suggesting a tight regulation of immune cells that interact with brain ECs in mouse models. Intriguingly, depression due to chronic stress alters BBB integrity in animal models, allowing the passage of monocytes and IL-6 from the circulation, and raising the question of whether compromised BBB integrity and depression may indeed be linked148. Interestingly, brain ECs have phagocytic capacity149, and microvascular ECs of the spinal cord can phagocytize myelin debris and recruit macrophages in vivo149, raising the question of whether specialized brain ECs may process antigens and promote brain inflammation in neurological diseases with an inflammatory component. Indeed, even though BBB ECs have low rates of pinocytosis (suggesting that this is not the main route for extracellular antigens to be acquired), they can present antigens on MHC-I and express MHC-II under inflammatory conditions150,151, which may facilitate adaptive immune responses in the brain by promoting T cell activation and potentially allowing antigen-specific T cells to enter the brain. scRNA-seq analyses of mouse and human brains provided further insights into the regional heterogeneity of ECs in the brain, in particular in the context of

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ageing and age-related neurodegenerative disease (Fig. 2). For example, brain ECs from hippocampi of aged mice upregulate the expression of VCAM1 in a vascular bed–specific pattern150. Indeed, venous and arterial VCAM1+ ECs expressed Tnfrsf1a, Il1r1, Il6ra and Il6st (generally considered to be pro-inflammatory), whereas venous VCAM1+ ECs additionally upregulated genes involved in immune cell infiltration, differentiation and antigen presentation (including Tspo, Lrg1 and B2m) and in pathways involved in TNF and NF-kB signalling151. This suggests that venous brain ECs are the most activated, and thus likely the immune cell-recruiting EC population in aged brains.

Another scRNA-seq study reported VCAM1 expression in a mixed mouse EC population (exhibiting arterial and venous features) but found that it was unaltered in brain ECs from aged brains compared with young brains152. However, aged capillary ECs had increased expression of genes involved in VCAM1-mediated immune cell migration153. Moreover, IFNγ response genes were downregulated in aged arterial and venous ECs compared with young controls, TLR-signalling was upregulated in aged arterial and venous–capillary ECs, and interleukin signalling was predominantly upregulated in aged capillary, venous and capillary–venous ECs154, suggesting a large heterogeneity in inflammatory signalling in ECs from different parts of the aged brain vasculature.

Other scRNA-seq studies document that ageing affects immunomodulation by capillary ECs by upregulating pathways involved in immune cell recruitment to the BBB, but also in innate immunity, TGFβ signalling and antigen processing155, or that ECs from aged mouse brains upregulate the expression of Cxcl12 (REF 156) (encoding a chemotactic ligand for CXCR4-expressing cells157) and Cd9 (REF 158) (encoding a surface protein that promotes the adhesion of immune cells to VCAM1 and ICAM1 [REF 159]). In the entorhinal cortex of patients with Alzheimer disease, ECs upregulated genes involved in the regulation of cytokine secretion and inflammation, including HLA-E (encoding a known natural killer cell modulator), MEF2C and NFKBIA158, indicating that ECs from brain regions affected by Alzheimer disease have a stronger inflammatory signature than brain ECs from age-matched healthy controls. These conflicting reports suggest that ECs from aged brains generally display immunological features that are atypical for ECs from non-aged brains, with the activation of specific subpopulations of brain ECs that are likely to promote the recruitment and functional modulation of immune cells. However, it is unclear which subtypes of brain ECs are most affected by ageing.

Conclusion

We have described the immunomodulatory functions of many different subsets of ECs, which we propose to collectively refer to as ‘IMECs’. The findings discussed herein suggest that (1) IMECs in tissues that are infiltrated by immune cells have specific immune cell-recruiting properties, a feature that can be induced by chronic inflammatory stimuli in non-lymphoid tissues; (2) IMECs in the lung and liver not only promote immune homeostasis but also mediate a careful balance between tolerance and inflammation (their role in immunomodulation may be partially determined by their anatomical location); (3) IMECs in the kidney and liver closely interact with resident immune cells, which may allow swift responses to circulating immune complexes; and (4) IMECs of immune privileged tissues such as the healthy brain form a tight and low immunomodulatory barrier to minimize infiltration of the tissue parenchyma. The capacity of IMECs to facilitate immune homeostasis might be more diverse than realized to date, and appears to depend on the specific subpopulation of ECs in a given tissue and their location in the vascular bed, and may change with age and in response to infection and disease.

However, there are a number of important outstanding questions. For example, it remains to be determined whether IMECs in tumours are tolerogenic or immunostimulatory, and whether they can be rendered more immunostimulatory by promoting their antigen-presenting function. If so, how could this be achieved? Does antigen presentation by IMECs in specific (which?) contexts, organs or conditions promote inflammation or tolerance? And when is antigen specificity a prerequisite for efficient immune cell migration159–160? Is the repertoire of antigens (presented by semi–professional antigen-presenting ECs) unique or generic compared with that of professional APCs? How important are IMECs as semi-professional APCs, considering their abundance compared with professional APCs? What is the main mechanism of antigen uptake for the different subtypes of IMECs? Does the apical–basolateral polarity of ECs affect antigen uptake from the circulation or tissue parenchyma? A related question is whether apically expressed MHC and adhesion molecules, which are the first molecules to which recruited T cells bind161, facilitate a sufficiently long interaction between the T cell and the IMEC to allow immunomodulation. Another question is whether some of these molecules are redistributed basolaterally and thereby prolong the duration of IMEC–T cell interaction. What is the contribution of IMECs interacting with perivascular immune cells to tissue immune homeostasis? And adding another layer of complexity, what is the relevance of bone marrow-derived endothelial progenitor cells, which might be recruited to replace injured IMECs162, and do these acquire tissue-specific immunomodulatory features similar to those of pre-existing IMECs? Do IMECs develop a form of trained immunity, as observed in in vitro experiments with human aortic ECs163–165? EC metabolism affects interferon-stimulated gene expression in ECs via effects on gene methylation, raising the question of how EC metabolism regulates IMEC function across tissues166. Are IMECs polarized towards a pro-inflammatory or an anti-inflammatory phenotype in a tissue-specific manner upon priming by specific pathogen-associated molecular patterns? What are the mechanisms of HEV biogenesis in non-lymphoid tissues? And how do HEV’s regulate immunity beyond immune cell recruitment?

The observation that subsets of ECs are involved in immune cell recruitment and vascular inflammation is not novel, but the concept that specific subpopulations of ECs are non-haematopoietic partners in an active immune response is an emerging concept, raising the translationally important question of whether the immunomodulatory capacity of IMECs can be targeted for immunotherapeutic purposes.
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