IMMUNOMODULATION BY ENDOTHELIAL CELLS - PARTNERING UP WITH THE IMMUNE SYSTEM?

Jacob Amersfoort[1], Guy Eelen[1*] & Peter Carmeliet [1-3&]*

(1) Laboratory of Angiogenesis & Vascular Metabolism, VIB & Department of Oncology, KU Leuven, Leuven, Belgium; (2) State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, P. R. China; (3) Laboratory of Angiogenesis and Vascular Heterogeneity; Department of Biomedicine, Aarhus University, Aarhus 8000, Denmark; *These authors contributed equally

&: Editorial correspondence:

P. Carmeliet, M.D., Ph.D.
Laboratory of Angiogenesis & Vascular Metabolism
Center for Cancer Biology
VIB, KU Leuven, Campus Gasthuisberg, Herestraat 49,
B-3000, Leuven, Belgium
tel: 32-16-34.57.72
e-mail: peter.carmeliet@kuleuven.be

ABSTRACT (129/150 WORDS)

Blood vessel endothelial cells (ECs) have been long 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 regards 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- 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.

Keywords: endothelial cells, immunity, single cell RNA sequencing, immunotherapy.

INTRODUCTION

Blood vessels had long been viewed as passive bystander conduits, with their sole function being the supply and drainage of blood to and from organs. Whereas lymph vessels are known to regulate various aspects of immunity[1,2], a potentially similar role for blood vessels has not received sufficient attention to date. Interestingly, endothelial cells (ECs), the cells that line blood vessels, share a common ancestor with immune cells (Box 1), intuitively supporting a role for ECs in immune responses.

Research from >100 years ago showed that ECs from the sinusoids of the liver, spleen and other organs can act as scavenger ECs (SECs), complementing the activity of macrophages in eliminating circulating waste macromolecules[3,4]. Indeed, SECs were proposed 10 years ago to be "an integral component of the innate immune system"[3], and like immune cells, liver sinusoidal ECs (LSECs) in rats can arise from bone marrow precursors in response to liver injury and during liver regeneration[3]. In addition, a combined single cell RNA-sequencing (scRNA-seq) and single cell Assay for Transposase-Accessible Chromatin-sequencing (scATAC-seq) study identified an "immune cell-like EC (EndICLT)" subpopulation among mouse aortic ECs, which is induced by disturbed blood flow. Induction of EndICLT marker genes was confirmed *in vitro* in human aortic ECs under disturbed flow-mimicking conditions[5]. In addition, it was found that during mouse embryonic development, aortic ECs can bud off from the ventral aorta and transition into hematopoietic cells; this was in part dependent on the transcription factor RUNX1[6]. Moreover, adult mouse ECs can be reprogrammed *in vivo* into hematopoietic stem cell-like cells through transient expression of the transcription factors FOSB, GFIL, RUNX1 and SPIl, and vascular-niche derived angiocrine factors[7].

Emerging evidence indicates that subsets of ECs in different tissues and organs exert immunomodulatory activities beyond their well-known role in allo-immunity, immune cell recruitment, immune tolerance and vascular inflammation[8–10]. Furthermore, several subtypes of ECs have been shown to display features that are typical of immune cells. These include the expression of co-stimulatory and

co-inhibitory receptors[11], the capacity to induce apoptosis in other cells (for example, they have been shown to kill ovarian tumor-homing cytotoxic T cells via FAS-ligand in human co-cultures and mice[12]), secretion of cytokines and acting as (semi-professional) antigen-presenting cells (APCs). They can also act as phagocytes and scavengers of circulating waste macromolecules and participate in efferocytosis [4,11–14]. Notably, immunomodulation by ECs can be influenced by cytokines, such as IL-35[15] and IL-17A[16]. Given the fact that ECs are among the first cells to come into contact with circulating pathogens and are the first cells that immune cells interact with when invading tissue parenchyma, they are strategically ideally positioned as first-line defense system to participate in immune responses.

In this Perspective, we first provide an overview of some of the well-known 'traditional' immunomodulatory functions of ECs, such as immune cell recruitment and semi-professional antigen presentation. We then examine recent advances in our understanding of the context-dependent role of ECs in immunomodulation in different organs, which are mainly based on scRNA-seq analyses. These studies indicate that immunomodulation by specific subsets of ECs, which we collectively refer to as immunomodulatory ECs (IMECs), can have a prominent role in tissue-specific immunity, as well as in cancer, neurodegeneration and infectious diseases such as coronavirus disease 2019 (COVID-19). Some of these IMECs may have constitutive immunomodulatory activities (such as LSECs), while other IMECs may refer to (transitory) plastic phenotypes, induced by particular contextual conditions (such as EndICLTs).

[H1] Immune cell recruitment by ECs

In the late 90s and early 2000s, ECs were discovered to function as local gatekeepers of immunity[8]. By interacting with circulating innate and adaptive immune cells and controlling their extravasation from the circulation into the tissue parenchyma, ECs can indeed control tissue and lymph node (LN) inflammation[11,17]. This process involves the differential expression of adhesion molecules (such as vascular

cell adhesion molecule 1 (VCAM1)), selectins (such as E-/P-selectin), addressins (such as peripheral node addressin (PNAd), mostly in mucosal and lymphoid tissue) and chemokines (such as CCL2/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[11,18]. EC activation can be induced by cytokines such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor (TNF), but also pathogen associated molecular patterns (PAMPs), 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[21]. Some aspects of immune cell recruitment by ECs might differ between species (as is also the case for antigen presentation (see below and box 2)).

[H1] Antigen-presentation by ECs

Some EC subtypes are considered semi-professional APCs since they express genes involved in antigen capture, processing and presentation. For example, human renal vascular endothelial cells express the major histocompatibility complex class II (MHC-II) surface molecule HLA-DR, which allows them to present antigen to $CD4^+$ T cells[22–24], and *in vitro* experiments showed that human umbilical vein ECs can activate allogenic T cells[22–25]. However, unlike professional APCs (such as dendritic cells (DCs)), ECs generally do not express the surface receptors CD80 and CD86[26], which bind to CD28 on naïve T cells and are required for their activation. ECs therefore primarily activate antigen-experienced T cells, although experiments in mice have shown that naïve T cells can also be activated by ECs in the context of alloimmunity[27,28]. Importantly, not all molecules/processes related to APC function in ECs are conserved between species[29] (Box 2). Interferon (IFN)γ and TNF induce immunomodulatory processes in human and mouse ECs *in vitro*, including antigen uptake, processing and presentation[9,10]. Antigen presentation

and immune cell recruitment by ECs contribute to allo-immunity and kidney/heart transplantation failure, for example through $CD8^+$ T cell-induced lysis of ECs in the donor tissue[8,30–33]. Moreover, antigen presentation by human ECs has been implicated in autoimmune diseases such as rheumatoid arthritis[34].

There are estimated to be > 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 intra- and extracellular antigen depending on the EC subtype and activation status[9,36]. For the presentation of intracellular antigen by ECs, nitric oxide[37] and IFNγ can induce a modified proteasome[38,39], called the immunoproteasome, which facilitates antigen degradation and antigen loading[39]. ECs share many features with professional APCs, but differ from them in other aspects (Table 1). For instance, ECs are exposed to shear stress[40], which has been found to increase intercellular adhesion molecule-1 (ICAM1) expression[41–43]. These bind to T cell integrins, which are capable of increasing T cell receptor-signaling[44]. Moreover, shear stress increases the binding of selectins[45–47], upregulates E-selectin expression in response to IL-1β[48], 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 signaling, co-inhibitory molecule expression and T cell proliferation in the context of antigen presentation by ECs[49]. The role of non-conventional MHC-molecules such as MR-1 (activating mucosal associated invariant T cells[50]) and BTN3A1 (presenting phospho-antigens to $V\gamma9V\delta2^+$ T cells[51]) in antigen presentation in ECs have yet to be determined.

[H1] TISSUE -SPECIFIC IMMUNOMODULATION BY ECS

Studies from the last two decades examined possible roles of ECs in immunomodulation at the bulk population level[11,18,35,52–54]. 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[55]. Single-cell studies have now allowed to obtain deeper insights into the role of EC immunomodulation in: (i) the recruitment and homing of immune cells to lymph nodes; (ii) the modulation of immunity to external challenges in the liver and lung; (iii) the detection and clearance of immune complexes in the liver and kidney; and (iv) the shielding of the brain tissue parenchyma from immune cell invasion in healthy conditions.

[H2] LYMPHOID ORGANS

Secondary lymphoid organs, such as lymph nodes (LNs) and Peyer's 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 system interact[56]. LNs contain a vascular bed with a heterogeneous composition of ECs that line arterioles, capillaries and venules. Notably, LNs also contain high endothelial venules (HEVs), these are a subtype of post-capillary venules (PCVs), which are lined by high (tall and plump) ECs that are specialized in recruiting immune cells such as monocytes, plasmacytoid DC precursors, neutrophils, B cells and T cells[17,57–59]. Naïve T cells in the circulation home to LNs, 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, GlyCAM-1 or MAdCAM-1 containing the 6-sulfo sialyl Lewis X glycan modification. These modified adhesion molecules can be detected by antibodies binding PNAd, like MECA-79[60–62]. A combination of addressins and chemokines such as C-C Motif Chemokine Ligand (CCL)-21 facilitates the capture and tethering of naïve T cells on HEVs and promote their extravasation (Figure 1a) [17]. HEVs are extensively remodeled upon infection and the subsequent expansion of draining LNs[17], 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 for immunomodulation by the ECs. For T cells, which can reside in LN 'pockets' in close proximity to HEVs[17], 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 trans-endothelial migration of immune cells across conventional PCVs, which are the primary site of immune cell recruitment in many organs, is rapid[63,64,65] (for example, 6 min. for mouse neutrophils *in vivo*[66]), which limits sustained interactions with ECs. In the liver, lungs and kidneys, however, immune cell recruitment primarily occurs in capillaries which are often only a few μm in diameter[63,67]. This causes immune cells to crawl, slows down extravasation and prolongs interactions with ECs, potentially allowing for immunomodulation by ECs.

The characterization of HEVs at single cell resolution under inflammatory conditions has strengthened the concept that HEVs can modulate immune cells (Figure 1d). Indeed, scRNA-seq analysis of enriched mouse MECA-79$^+$ HEVs from LNs, 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 such as DCs[69]. Activated HEVs from oxalozone-exposed mice also express higher levels of macrophage migration inhibitory factor (MIF), which regulates context-dependent M1/M2 macrophage polarization[70,71], and thrombospondin-1 (TSP-1), 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 LNs implied that non-HEV ECs can recruit myeloid cells to LNs during inflammation in a MECA-79 independent, but P- and E-selectin-dependent manner[74], implying that not only HEVs are important for (myeloid) immune cell recruitment during inflammation (Figure 1b). Single cell studies in mouse and

human tumors further revealed that there is no clear phenotypic separation between HEVs and (post-capillary) venous ECs in tumors, which express a selected set of canonical and non-canonical HEV markers [75–77].

Interestingly, a combination therapy of anti-VEGF therapy (which facilitates vessel normalization) and anti-PD-L1 immunotherapy promotes HEV formation and T cell recruitment, and improves anti-tumor immunity in preclinical tumor models[78]. Similarly, the treatment of mice with anti-PD1 in combination with delivery of vascular-targeted LIGHT proteins that induce non-canonical NF-κB signaling, which is required for differentiation of ECs into the HEV phenotype, induces HEV biogenesis and improves tumor immunity and immunotherapy in preclinical tumor models[79,80] (Figure 1c). Thus, in addition to the established function of HEVs in immune cell trafficking to LNs during infections, HEVs may also have direct immunomodulatory effects. Further insight into this additional immunomodulatory potential and their extra-lymphatic biogenesis during (chronic) inflammation, cancer and other diseases may offer new immunotherapeutic opportunities for these conditions.

[H2] ORGANS CONTROLLING IMMUNITY VS. TOLERANCE TO EXTERNAL DANGER

Several organs, such as the liver, intestines, lung and skin, are exposed to airborne or nutrient-derived antigens, pathogens, toxins and to their microbiome, as well as microbiome-derived antigens (Figure 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.

[H3] 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 liver sinusoidal ECs (LSECs).

LSECs are equipped with a repertoire of molecules for the detection and uptake of extracellular antigens (microbial products, viruses), including the Toll-like Receptors (TLRs)1-4, TLR6, TLR8, TLR9[81,82] and scavenger receptors such as the C-type lectin receptor mannose receptor[83,84]. In mice, LSECs take up and cross-present extracellular antigen 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 only at low levels) the costimulatory receptors CD80/CD86, which are necessary for the activation of naïve T cells[85–87]. Similarly, exogenous antigen, acquired through mannose receptor-mediated endocytosis and presented on MHC-II molecules to naïve $CD4^+$ T cells, induces tolerance by promoting regulatory T (T_{reg}) cell differentiation (Figure 1e)[88,89]. Additionally, LSECs are also involved in Fc-receptor-mediated phagocytosis and degradation of (primarily large) antibody/antigen immune complexes from the circulation[3,90] (Figure 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 VAP-1, whereas $CD8^+$ T cell extravasation into the liver is primarily mediated by ICAM1[91–93]. Since LSECs exhibit zone-dependent heterogeneity in liver lobules[94,95], these findings raise the question 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 hepatic zones[96] due to MYD88-dependent signaling in LSECs, which is induced by gut commensal bacteria and changes the composition of the LSEC glycocalyx layer and hence the gradients of chemokines (such as CXCL9) binding to components of the glycocalyx (such as glycosaminoglycans) (Figure 1g). The resulting periportal 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 defense.

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* (encoding an inhibitory receptor) and *STAB1* (encoding stabilin-1) and of genes involved in innate immunity, phagocytosis and leukocyte activation, whereas periportal ECs exhibit a TNF activation signature and express other immunomodulatory genes[95]. Yet, a paired-cell RNA-seq study of livers from healthy mice, in which mRNA from pairs of ECs attached to hepatocytes were sequenced and gene expression from one cell type was used to infer the tissue coordinates of the cell pair, reports opposite findings, indicating low levels of *STAB1* transcription in central vein LSECs[94]. Moreover, this report identified close interactions between LSECs and Kupffer cells (liver resident macrophages) through colony stimulating factor-1 (CSF-1)/CSF-1 receptor and CD93/C1qa signaling[94] (Figure 1g). Overall, although all these studies documented regional LSEC heterogeneity and interactions between LSECs and immune cells, further protein-level validation is needed to confirm their relevance.

LSECs also affect disease outcome. For example, LSECs present cancer cell-derived apoptotic bodies to naïve $CD8^+$ T cells. However, since LSECs act as semi-professional APCs, they impair the differentiation of naïve $CD8^+$ T cells into cytotoxic effector T cells, which are capable of killing cancer cells, thereby hampering tumor immunity[1]. It was shown that breaking LSEC-induced immune tolerance (using nanoparticles to deliver melittin, a host defense 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[98]. In mouse models of hepatocellular carcinoma, malignant hepatocyte-derived VEGF induces the expression of the EC-specific transmembrane protein PLVAP in LSECs, which promotes the recruitment of $FOLR2^+$ immunosuppressive tumor-associated macrophages and the creation of an immunosuppressive niche by interacting with T_{reg} cells[99]. This suggests that LSECs form a communication hub in the liver tumor microenvironment that promotes immunosuppression and thereby facilitates tumor growth (Figure 1h).

LSECs can also promote excessive inflammation in mice and humans and contribute to organ damage in conditions such as autoimmune hepatitis[100,101] and fibrosis[102], suggesting that immunomodulation by LSECs is critical for maintaining an immunological balance and tissue homeostasis in the liver. Further, a scRNA-seq study of healthy and cirrhotic human livers showed that the latter contained a disease-specific EC population in the fibrotic niche[102], which was enriched in *ACKR1* transcripts (Figure 1i)[102], encoding the atypical chemokine receptor 1 (ACKR1). This chemokine receptor is primarily expressed by PCV ECs (and small venule ECs[103]), and transports basal chemokines for presentation at the luminal surface of ECs and in paracellular junctions, where it regulates different stages of immune cell diapedesis[104] and recruitment[105]. Moreover, *in silico* analyses predicted that ACKR1$^+$ ECs interact with disease-specific macrophages via multiple chemokines (such as CXCL12 and CCL2) and the macrophage differentiation factors GAS6 and PROS1[102]. This suggests that ACKR1$^+$ ECs might recruit disease-specific immune cells, and raises the question whether liver ECs might be therapeutic targets to treat liver cirrhosis. In mice with experimentally induced portal hypertension, LSECs express lower levels of MHC-I and MHC-II molecules[106], suggesting that immune responses in the liver may be altered in this disease. Finally, a scRNA-seq study in aged mice unveiled decreased *MRC1* expression (encoding the C-type lectin receptor CD206) in LSECs, which might contribute to their decrease in endocytic capacity with age[107]. However, *in situ* RNA staining for *MRC1* and the classical LSEC marker *PECAM1* (encoding CD31) in the same study showed that the number of *MRC1*-expressing LSECs actually increases with age in mice, raising the question whether LSECs in aged individuals have a reduced or similar immunomodulatory potential. Overall, LSECs differ from ECs in other tissues by their constant exposure to dietary and pathogen-derived antigens, exert a predominantly tolerogenic APC function, and show zonal heterogeneity.

[H3] THE LUNG

The lung is highly vascularized with a specialized composition of ECs, largely consisting 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[108]. Immunomodulation by ECs might play a more important role in the lung than originally anticipated.

Indeed, compared to 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[109]. Moreover, subsets of lung ECs express MHC-II, and in humans this feature appears to be restricted to capillary ECs[75,110]. A recent scRNA-seq study revealed that human bronchial ECs form a transcriptomically distinct population from alveolar ECs, although genes involved in immunomodulation do not appear to be their most distinguishing feature[111]. Another single cell study suggested that human alveolar capillary ECs can be divided in two populations, based on their transcriptome and location, where ECs termed aerocytes (which are located in close proximity 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[112], 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 (commensal) microbiota (Figure 2c). Indeed, a single cell analysis of alveolar cell populations (conserved in humans, mice, rats and pigs) predicted capillary ECs to be the most responsive cell type to VEGF (released primarily by alveolar type 1- and secretory epithelial cells[113]). Given the immunosuppressive effects of VEGF[114], the above finding raises the question whether VEGF signaling

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 progression in lung cancer. Tumor ECs (TECs) from individuals with untreated, non-metastatic non-small cell lung cancer (NSCLC) of the squamous cell or adeno-carcinoma subtype exhibit a decreased expression of genes encoding ICAM1, the chemokines CCL2 and CCL18, the cytokine IL6 and HLA-I/HLA-II[115], suggesting an immunosuppressive environment[116]. Additionally, TECs of human and mouse lungs show elevated expression of genes encoding FAS-L, a cell death regulator capable of inducing cell death in cytotoxic T cells[12], and of co-inhibitory molecules such as PD-L1, further indicating an immunosuppressive role (Figure 2a)[117]. Another single cell study of human and mouse lung tumors illustrated a complex immunomodulatory gene signature[75]. In line with earlier studies, lung capillary TECs expressed lower levels of immunomodulatory genes (involved in antigen presentation and processing) than peritumoral capillary ECs, suggesting that certain TEC subpopulations might become more tolerogenic[75]. However, tumors had fewer capillaries, which suggests that further research is required to investigate the exact immunomodulatory role of lung capillary TECs[75]. Further, mice with a deficiency of MHC-II in non-hematopoietic cells had fewer T_{reg} cells in the lung and a lower pulmonary metastasis burden in lung tumor models[110], which may suggest that antigen presentation by pulmonary ECs contributes to immune tolerance in lung cancer, although EC-selective knock-out approaches are required to confirm this. However, another population of activated PCV lung ECs that was enriched in human NSCLC and mouse lung tumors, 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 tumor immunity[75]. Notably, mass cytometry revealed high surface expression of HLA-DRA on healthy capillary

lung ECs, which was comparable to immune cells in general. This finding requires further functional validation, but highlights the immunomodulatory potential of these ECs as non-professional APCs[75].

The role of lung ECs has also been investigated in various infection models. For example, in a mouse model of *P. berghei*-induced malaria, lung ECs were shown to cross-present malaria parasite antigens to $CD8^+$ T cells (this was also shown *in vitro*) in response to stimulation by IFNγ, which is presumably secreted by $CD8^+$ T cells (and possibly $CD4^+$ T cells and NK cells). This process is associated with vascular leakage and lung damage (Figure 2b)[118], indicating that antigen presentation by lung ECs can have detrimental effects. Vascularized lung-on-chip models allow to investigate the role of lung ECs in infections such as COVID-19. These showed that lung ECs underlying epithelial cells can be directly infected with SARS-CoV-2 and contained viral RNA (however, without signs of active viral replication), and infected ECs exhibited a decreased barrier integrity[119]. In aged mice, pulmonary capillary ECs have been shown to upregulate various cytokine transcripts (such as *IL1b*, *TNFa*, *TGFb1*)[120], which suggests that capillaries might contribute to lung diseases that are more prevalent in older individuals, such as chronic obstructive pulmonary disease and lung cancer[121], and possibly contribute to the severity of COVID-19[122]. 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 lung[122]. 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 lung[119].

Similarly, in influenza infection, ECs may contribute to the cytokine storm that characterizes severe infection[123]. Viral replication in mouse ECs has been shown for specific influenza strains[124], and this might impair the barrier function of the lung epithelium. Hence, viral replication in specific subtypes of ECs, such as capillary ECs, might induce viral antigen presentation and contribute to a rapid recall

response of intra- or perivascular memory T cells[125]. Together, emerging evidence indicates that pulmonary ECs are involved in immune responses, but whether they promote immunity (and potential tissue pathology in infections) or tolerance appears to be contextual and requires further study.

[H2] 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 nephron[126,127]. 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 system[126]. 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 ECs[128,129]. 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 validated (Figure 2g)[130]. Interestingly, medullary capillary ECs from dehydrated mice, which are exposed to non-physiologically high osmolarities, lower their transcriptional response to IFN-β[130], indicating that different osmolarities 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 to other ECs, glomerular ECs have a particularly thick filamentous glycocalyx 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 glycocalyx exposes surface molecules on ECs that facilitate the extravasation of immune cells into the glomeruli (Figure 2d)[126,131,132]. This can contribute to immune cell-mediated damage of glomeruli when immune cells such as neutrophils infiltrate the glomeruli and release their granules[126]. 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 inflammatory response if appropriately stimulated (Figure 2e)[4].

Immunomodulation by renal ECs is of particular interest in the context of organ transplantation. Renal microvascular ECs are frequently targets of donor-specific antibodies (DSAs) that bind to HLA molecules expressed by the transplanted kidney, and ECs contribute to allo-immunity by upregulating HLA-II genes after transplantation (Figure 2f)[133,134]. A recent study of transplanted human kidneys documented a not further specified subpopulation of donor ECs in the transplanted kidney that showed signs of activation[135] (suggesting that it is a target of DSA-mediated rejection) and an upregulation of genes involved in phagocytosis[135], which may indicate antibody uptake. Also, under stress conditions, renal ECs (subtype to be specified) produce transforming growth factor-beta (TGF-β)[136] and can secrete large amounts of interleukin-6 (IL-6)[137]. These cytokines can promote the differentiation of naïve $CD4^+$ T cells into either immunosuppressive T_{reg} cells (when only TGF-β is present) or pro-inflammatory T helper-17 (T_H-17) cells (when TGF-β plus IL-6 are present)[138]. Since antigens presented by MHC-II molecules on renal ECs can skew $CD4^+$ T cell differentiation towards either T_{reg} or T_H-17 cells[139–141], the inflammatory context that renal ECs are exposed to might 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 prior to transplantation may allow to tweak EC-mediated immunomodulation in such a

384 way that allo-immunity is decreased and transplantation success increased. Finally, in Wilms tumors, a
385 cancer affecting the kidneys, renal TECs upregulate *ACKR1* transcription[142]. Whether the potential for
386 immune cell recruitment by ACKR1$^+$ TECs can be exploited by tuning additional TEC populations to ac-
387 quire ACKR1 expression to stimulate tumoricidal immune cell infiltration might be of interest as anti-
388 cancer therapy, given the generally immunosuppressive features of TECs.

389 ## [H2] THE BRAIN

390 In healthy conditions, the brain is poorly infiltrated by immune cells due to the low expression of ad-
391 hesion molecules by the specialized capillary and PCV ECs of the blood-brain-barrier (BBB)[143] and the
392 abundance of tight junctions between these ECs . Brain ECs thus exhibit a larger level of immune anergy
393 and contribute to the maintenance of the immune privileged state of the brain[54]. Unlike liver and renal
394 ECs, BBB ECs lack fenestrations and form continuous intercellular junctional complexes, limiting para-
395 cellular leakage of molecules from the circulation into the brain. Further, BBB ECs not only express low
396 levels of adhesion molecules (such as ICAM-1), but also express lower levels of cytokines and chemo-
397 kines (such as IL-8, CCL2), regulated in part by astrocyte-derived sonic hedgehog, which, via hedgehog
398 receptors, induces immune quiescence in ECs, impairing immune cell migration[144].

399 However, in models of infection or inflammatory disease, BBB ECs upregulate adhesion mole-
400 cules (such as E-/P-selectins) and chemokines (such as CXCL1), thereby promoting immune cell infiltra-
401 tion and inflammation in the brain (Figure 2h)[53,145,146]. For example, after transmigration, extravasated
402 monocytes differentiate into T_H17-polarizing DCs in response to brain EC-derived granulocyte macro-
403 phage colony stimulating factor (GM-CSF) and TGF-β[147], suggesting a tight regulation of immune cells
404 that interact with brain ECs in mouse models. Intriguingly, depression due to chronic stress alters BBB
405 integrity in animal models, allowing the passage of monocytes and IL-6 from the circulation, and raising

406 the question whether compromised BBB integrity and depression may indeed be linked[148]. Interest-
407 ingly, brain ECs have phagocytotic capacity[149] and microvascular ECs of the spinal cord can phagocytose
408 myelin debris and recruit macrophages *in vivo*[14], raising the question whether specialized brain ECs may
409 process antigen and promote brain inflammation in neurological diseases with an inflammatory com-
410 ponent. Indeed, even though BBB ECs have low rates of pinocytosis (suggesting that this is not the main
411 route for extracellular antigens to be acquired), they can present antigens on MHC-I and express MHC-
412 II under inflammatory conditions[150,151], which may facilitate adaptive immune responses in the brain
413 by promoting T cell activation and potentially allowing antigen-specific T cells to enter the brain.

414 scRNA-seq analyses of mouse and human brains provided further insights into the regional het-
415 erogeneity of ECs in the brain, in particular in the context of aging and age-related neurodegenerative
416 disease (Figure 2i). For example, brain ECs from hippocampi of aged mice upregulate the expression of
417 VCAM1 in a vascular bed-specific pattern[152]. Indeed, venous- and arterial VCAM1$^+$ ECs expressed
418 *Tnfrsf1a*, *Il1r1*, *Il6ra* and *Il6s* (generally considered to be pro-inflammatory), whereas venous VCAM1$^+$
419 ECs additionally upregulated genes involved in immune cell infiltration, differentiation and antigen
420 presentation (including *Tspo*, *Lrg1*, *B2m*) and in pathways involved in TNF and NF-κB signaling[152]. This
421 suggests that venous brain ECs are the most activated, and thus likely the immune-cell-recruiting EC
422 population in aged brains.

423 Another scRNA-seq study reported VCAM1 expression in a mixed mouse EC population (exhib-
424 iting arterial- and venous features) but found that it was unaltered in brain ECs from aged brains com-
425 pared to young brains [153]. However, aged capillary ECs had an increased expression of genes involved
426 in VCAM1-mediated immune cell migration[153]. Moreover, IFNγ response genes were downregulated in
427 aged arterial and venous ECs compared to young controls, TLR-signaling was upregulated in aged arte-

rial and venous-capillary ECs, and interleukin-signaling was predominantly upregulated in aged capillary, venous and capillary-venous ECs[153], suggesting a large heterogeneity in inflammatory signaling in ECs from different parts of the aged brain vasculature.

Yet other scRNA-seq studies document that aging affects immunomodulation by capillary ECs by upregulating pathways involved in immune cell recruitment to the BBB, but also in innate immunity, TGF-β signaling and antigen processing[145], or that ECs from aged mouse brains upregulate the expression of *Cxcl12*[154] (encoding a chemotactic ligand for CXCR4-expressing cells[155]) and *Cd9*[154] (encoding a surface protein that promotes the adhesion of immune cells to VCAM1 and ICAM1[156]). In the entorhinal cortex of patients with Alzheimer's disease, ECs upregulated genes involved in the regulation of cytokine secretion and inflammation, including *HLA-E* (encoding a known NK cell modulator), *MEF2C* and *NFKBIA*[157], indicating that ECs from brain regions affected by Alzheimer's 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 that are likely to promote the recruitment and functional modulation of immune cells. However, it is yet unclear which subtypes of brain ECs are most affected by aging.

[H1] Conclusion

Above, we described the immunomodulatory functions of many different subsets of ECs, which we propose to collectively refer to as IMECs. The findings discussed above suggest that: (i) 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; (ii) 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; (iii)

IMECs in the kidney and liver closely interact with resident immune cells, which may allow swift responses to circulating immune complexes; and (iv) IMECs of immune privileged tissues such as the healthy brain form a tight and low immune-modulatory 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, 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 tumors 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, conditions promote inflammation or tolerance? And when is antigen-specificity a prerequisite for efficient immune cell migration[158–160]? Is the repertoire of antigens (presented by semi-professional antigen-presenting ECs) unique or generic, compared to professional APCs? How important are IMECs as semi-professional APCs, considering their abundance compared to 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 that recruited T cells bind to[11], facilitate a sufficiently long interaction between the T cell and the IMEC to allow for immunomodulation. Another question is whether some of these molecules are redistributed baso-laterally 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 IMECs[3,161], and do these acquire similar tissue-specific immunomodulatory features

as pre-existing IMECs? Do IMECs develop a form of trained immunity, as observed in *in vitro* experiments with human aortic ECs[162–164]? EC metabolism affects interferon-stimulated gene expression in ECs via effects on gene methylation, raising the question how EC metabolism regulates IMEC function across tissues[165]. Are IMECs polarized towards a pro- or anti-inflammatory phenotype upon priming by specific PAMPs, in a tissue-specific manner? What are the mechanisms of HEV biogenesis in non-lymphoid tissues? And how do HEVs 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-hematopoietic partners in an active immune response is an emerging concept, raising the translationally important question whether the immunomodulatory capacity of IMECs can be targeted for immunotherapeutic purposes.

REFERENCES

1. Jackson, D. G. Leucocyte Trafficking via the Lymphatic Vasculature— Mechanisms and Consequences. *Front. Immunol.* **10**, (2019).
2. Jalkanen, S. & Salmi, M. Lymphatic endothelial cells of the lymph node. *Nat. Rev. Immunol.* **20**, 566–578 (2020).
3. Sørensen, K. K. *et al.* The scavenger endothelial cell: a new player in homeostasis and immunity. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **303**, R1217–R1230 (2012).
4. Stamatiades, E. G. *et al.* Immune Monitoring of Trans-endothelial Transport by Kidney-Resident Macrophages. *Cell* **166**, 991–1003 (2016).

This paper describes how renal endothelial cells transport immune complexes from the circulation to perivascular macrophages for optimal cooperative immune monitoring.

5. Andueza, A. *et al.* Endothelial Reprogramming by Disturbed Flow Revealed by Single-Cell RNA and Chromatin Accessibility Study. *Cell Rep.* **33**, 108491 (2020).

This study describes an endothelial immune cell-like type that arises under disturbed flow and expresses markers commonly associated with macrophages.

6. Boisset, J.-C. *et al.* In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature* **464**, 116–120 (2010).
7. Lis, R. *et al.* Conversion of adult endothelium to immunocompetent haematopoietic stem cells. *Nature* **545**, 439–445 (2017).
8. Pober, J. S. & Sessa, W. C. Evolving functions of endothelial cells in inflammation. *Nat. Rev. Immunol.* **7**, 803–815 (2007).
9. Lohse, A. *et al.* Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. *Gastroenterology* **110**, 1175–1181 (1996).
10. Wedgwood, J. F., Hatam, L. & Bonagura, V. R. Effect of interferon-γ and tumor necrosis factor on the expression of class I and class II major histocompatibility molecules by cultured human umbilical vein endothelial cells. *Cell. Immunol.* **111**, 1–9 (1988).
11. Carman, C. V. & Martinelli, R. T Lymphocyte–Endothelial Interactions: Emerging Understanding of Trafficking and Antigen-Specific Immunity. *Front. Immunol.* **6**, (2015).
12. Motz, G. T. *et al.* Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* **20**, 607–15 (2014).

13. Dini, L. *et al.* Phagocytosis of apoptotic bodies by liver endothelial cells. *J. Cell Sci.* **108**, 967–973 (1995).

14. Zhou, T. *et al.* Microvascular endothelial cells engulf myelin debris and promote macrophage recruitment and fibrosis after neural injury. *Nat. Neurosci.* **22**, 421–435 (2019).

15. Sha, X. *et al.* Interleukin-35 Inhibits Endothelial Cell Activation by Suppressing MAPK-AP-1 Pathway. *J. Biol. Chem.* **290**, 19307–19318 (2015).

16. Mai, J. *et al.* Interleukin-17A Promotes Aortic Endothelial Cell Activation via Transcriptionally and Post-translationally Activating p38 Mitogen-activated Protein Kinase (MAPK) Pathway. *J. Biol. Chem.* **291**, 4939–4954 (2016).

17. Ager, A. High Endothelial Venules and Other Blood Vessels: Critical Regulators of Lymphoid Organ Development and Function. *Front. Immunol.* **8**, (2017).

18. Georganaki, M., van Hooren, L. & Dimberg, A. Vascular Targeting to Increase the Efficiency of Immune Checkpoint Blockade in Cancer. *Front. Immunol.* **9**, 3081 (2018).

19. Dauphinee, S. M. & Karsan, A. Lipopolysaccharide signaling in endothelial cells. *Lab. Invest.* **86**, 9–22 (2006).

20. Liao, J. K. Linking endothelial dysfunction with endothelial cell activation. *J. Clin. Invest.* **123**, 540–541 (2013).

21. Muller, W. A. Leukocyte-Endothelial Cell Interactions in the Inflammatory Response. *Lab. Invest.* **82**, 521–534 (2002).

22. Scott, H., Brandtzaeg, P., Hirschberg, H., Solheim, B. G. & Thorsby, E. Vascular and renal distribution of HLA-DR-like antigens. *Tissue Antigens* **18**, 195–202 (1981).

23. Hancock, W. W., Kraft, N. & Atkins, R. C. The immunohistochemical demonstration of major histocompatibility antigens in the human kidney using monoclonal antibodies. *Pathology (Phila.)* **14**, 409–414 (1982).

24. Hart, D. N. *et al.* Localization of HLA-ABC and DR antigens in human kidney. *Transplantation* **31**, 428–433 (1981).

25. Hirschberg, H., Bergh, O. J. & Thorsby, E. Antigen-presenting properties of human vascular endothelial cells. *J. Exp. Med.* **152**, 249s–255s (1980).

26. Vandenberghe, P., Delabie, J., de Boer, M., De Wolf-Peeters, C. & Ceuppens, J. L. In situ expression of B7/BB1 on antigenpresenting cells and activated B cells: an immunohistochemical study. *Int. Immunol.* **5**, 317–321 (1993).

27. Kreisel, D. *et al.* Mouse Vascular Endothelium Activates CD8+ T Lymphocytes in a B7-Dependent Fashion. *J. Immunol.* **169**, 6154–6161 (2002).

28. Walch, J. M. *et al.* Cognate antigen directs CD8+ T cell migration to vascularized transplants. *J. Clin. Invest.* **123**, 2663–2671 (2013).

29. Pober, J. S., Merola, J., Liu, R. & Manes, T. D. Antigen Presentation by Vascular Cells. *Front. Immunol.* **8**, 1907 (2017).

30. Meehan, S. M. *et al.* Cytotoxicity and apoptosis in human renal allografts: identification, distribution, and quantitation of cells with a cytotoxic granule protein GMP-17 (TIA-1) and cells with fragmented nuclear DNA. *Lab. Investig. J. Tech. Methods Pathol.* **76**, 639–649 (1997).

31. Colvin, R. B. *et al.* Evaluation of pathologic criteria for acute renal allograft rejection: reproducibility, sensitivity, and clinical correlation. *J. Am. Soc. Nephrol. JASN* **8**, 1930–1941 (1997).

32. Jutte, N. H. P. M. *et al.* HUMAN HEART ENDOTHELIAL-CELL-RESTRICTED ALLORECOGNITION. *Transplantation* **62**, 403–406 (1996).

33. Al-Lamki, R. S., Bradley, J. R. & Pober, J. S. ENDOTHELIAL CELLS IN ALLOGRAFT REJECTION. *Transplantation* **86**, 1340–1348 (2008).

34. Turesson, C. Endothelial Expression of MHC Class II Molecules in Autoimmune Disease. *Current Pharmaceutical Design* vol. 10 129–143 https://www.eurekaselect.com/61971/article (2003).

35. Mai, J., Virtue, A., Shen, J., Wang, H. & Yang, X.-F. An evolving new paradigm: endothelial cells – conditional innate immune cells. *J. Hematol. Oncol.J Hematol Oncol* **6**, 61 (2013).

36. Limmer, A. *et al.* Efficient presentation of exogenous antigen by liver endothelial cells to CD8 + T cells results in antigen-specific T-cell tolerance. *Nat. Med.* **6**, 1348–1354 (2000).

37. Kotamraju, S. *et al.* Upregulation of immunoproteasomes by nitric oxide: Potential antioxidative mechanism in endothelial cells. *Free Radic. Biol. Med.* **40**, 1034–1044 (2006).

38. Foss, G. S. & Prydz, H. Interferon Regulatory Factor 1 Mediates the Interferon-γ Induction of the Human Immunoproteasome Subunit Multicatalytic Endopeptidase Complex-like 1. *J. Biol. Chem.* **274**, 35196–35202 (1999).

39. Murata, S., Takahama, Y., Kasahara, M. & Tanaka, K. The immunoproteasome and thymoproteasome: functions, evolution and human disease. *Nat. Immunol.* **19**, 923–931 (2018).

40. Givens, C. & Tzima, E. Endothelial Mechanosignaling: Does One Sensor Fit All? *Antioxid. Redox Signal.* **25**, 373–388 (2016).

41. Sheikh, S., Rainger, G. E., Gale, Z., Rahman, M. & Nash, G. B. Exposure to fluid shear stress modulates the ability of endothelial cells to recruit neutrophils in response to tumor necrosis factor-α: a basis for local variations in vascular sensitivity to inflammation. *Blood* **102**, 2828–2834 (2003).

42. Chiu Jeng-Jiann *et al.* Shear Stress Increases ICAM-1 and Decreases VCAM-1 and E-selectin Expressions Induced by Tumor Necrosis Factor-α in Endothelial Cells. *Arterioscler. Thromb. Vasc. Biol.* **24**, 73–79 (2004).

583 43. Walpola Piyal L., Gotlieb Avrum I., Cybulsky Myron I., & Langille B. Lowell. Expression of ICAM-1
584 and VCAM-1 and Monocyte Adherence in Arteries Exposed to Altered Shear Stress. *Arterioscler.*
585 *Thromb. Vasc. Biol.* **15**, 2–10 (1995).

586 44. Jankowska, K. I. *et al.* Integrins Modulate T Cell Receptor Signaling by Constraining Actin Flow at
587 the Immunological Synapse. *Front. Immunol.* **9**, (2018).

588 45. Finger, E. B. *et al.* Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature*
589 **379**, 266–269 (1996).

590 46. Paschall, C. D., Guilford, W. H. & Lawrence, M. B. Enhancement of L-Selectin, but Not P-Selectin,
591 Bond Formation Frequency by Convective Flow. *Biophys. J.* **94**, 1034–1045 (2008).

592 47. Lawrence, M. B., Kansas, G. S., Kunkel, E. J. & Ley, K. Threshold Levels of Fluid Shear Promote
593 Leukocyte Adhesion through Selectins (CD62L,P,E). *J. Cell Biol.* **136**, 717–727 (1997).

594 48. Huang, R. B. & Eniola-Adefeso, O. Shear Stress Modulation of IL-1β-Induced E-Selectin Expression
595 in Human Endothelial Cells. *PLOS ONE* **7**, e31874 (2012).

596 49. Tinoco, R., Otero, D. C., Takahashi, A. & Bradley, L. M. PSGL-1: A New Player in the Immune Check-
597 point Landscape. *Trends Immunol.* **38**, 323–335 (2017).

598 50. Gold, M. C., Napier, R. J. & Lewinsohn, D. M. MR1-restricted mucosal associated invariant T (MAIT)
599 cells in the immune response to Mycobacterium tuberculosis. *Immunol. Rev.* **264**, 154–166 (2015).

600 51. Rigau, M. *et al.* Butyrophilin 2A1 is essential for phosphoantigen reactivity by γδ T cells. *Science*
601 **367**, (2020).

602 52. Shetty, S., Lalor, P. F. & Adams, D. H. Liver sinusoidal endothelial cells — gatekeepers of hepatic
603 immunity. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 555–567 (2018).

604 53. Sonar, S. A. & Lal, G. Blood-brain barrier and its function during inflammation and autoimmunity.
605 *J. Leukoc. Biol.* **103**, 839–853 (2018).

606 54. Spadoni, I., Fornasa, G. & Rescigno, M. Organ-specific protection mediated by cooperation be-
607 tween vascular and epithelial barriers. *Nat. Rev. Immunol.* **17**, 761–773 (2017).

608 55. Krausgruber, T. *et al.* Structural cells are key regulators of organ-specific immune responses. *Na-*
609 *ture* 1–7 (2020) doi:10.1038/s41586-020-2424-4.

610 56. Randolph, G. J., Ivanov, S., Zinselmeyer, B. H. & Scallan, J. P. The Lymphatic System: Integral Roles
611 in Immunity. *Annu. Rev. Immunol.* **35**, 31–52 (2017).

612 57. Bogoslowski, A., Butcher, E. C. & Kubes, P. Neutrophils recruited through high endothelial venules
613 of the lymph nodes via PNAd intercept disseminating *Staphylococcus aureus*. *Proc. Natl. Acad. Sci.*
614 **115**, 2449–2454 (2018).

615 58. Palframan, R. T. *et al.* Inflammatory Chemokine Transport and Presentation in HEV. *J. Exp. Med.*
616 **194**, 1361–1374 (2001).

59. Yoneyama, H. *et al.* Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules. *Int. Immunol.* **16**, 915–928 (2004).

60. Uchimura, K. *et al.* A major class of L-selectin ligands is eliminated in mice deficient in two sulfotransferases expressed in high endothelial venules. *Nat. Immunol.* **6**, 1105–1113 (2005).

61. Mitoma, J. *et al.* Critical functions of N -glycans in L-selectin-mediated lymphocyte homing and recruitment. *Nat. Immunol.* **8**, 409–418 (2007).

62. Kawashima, H. *et al.* N -acetylglucosamine-6- O -sulfotransferases 1 and 2 cooperatively control lymphocyte homing through L-selectin ligand biosynthesis in high endothelial venules. *Nat. Immunol.* **6**, 1096–1104 (2005).

63. Maas, S. L., Soehnlein, O. & Viola, J. R. Organ-Specific Mechanisms of Transendothelial Neutrophil Migration in the Lung, Liver, Kidney, and Aorta. *Front. Immunol.* **9**, (2018).

64. Yadav, R., Larbi, K., Young, R. & Nourshargh, S. Migration of leukocytes through the vessel wall and beyond. *Thromb. Haemost.* **90**, 598–606 (2003).

65. Proebstl, D. *et al.* Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. *J. Exp. Med.* **209**, 1219–1234 (2012).

66. Woodfin, A. *et al.* Junctional adhesion molecule-C (JAM-C) regulates polarized neutrophil transendothelial cell migration in vivo. *Nat. Immunol.* **12**, 761–769 (2011).

67. Aird William C. Phenotypic Heterogeneity of the Endothelium. *Circ. Res.* **100**, 158–173 (2007).

68. Browning, J. L. *et al.* Lymphotoxin-β Receptor Signaling Is Required for the Homeostatic Control of HEV Differentiation and Function. *Immunity* **23**, 539–550 (2005).

69. Kang, S. W. *et al.* Anti-CD137 Suppresses Tumor Growth by Blocking Reverse Signaling by CD137 Ligand. *Cancer Res.* **77**, 5989–6000 (2017).

70. Yaddanapudi, K. *et al.* Control of tumor-associated macrophage alternative activation by MIF. *J. Immunol. Baltim. Md 1950* **190**, 2984–2993 (2013).

71. Castro, B. A. *et al.* Macrophage migration inhibitory factor downregulation: a novel mechanism of resistance to anti-angiogenic therapy. *Oncogene* **36**, 3749–3759 (2017).

72. Li, Z., He, L., Wilson, K. & Roberts, D. Thrombospondin-1 inhibits TCR-mediated T lymphocyte early activation. *J. Immunol. Baltim. Md 1950* **166**, 2427–2436 (2001).

73. Veerman, K., Tardiveau, C., Martins, F., Coudert, J. & Girard, J.-P. Single-Cell Analysis Reveals Heterogeneity of High Endothelial Venules and Different Regulation of Genes Controlling Lymphocyte Entry to Lymph Nodes. *Cell Rep.* **26**, 3116-3131.e5 (2019).

This paper uses single cell RNA-sequencing to describe HEV heterogeneity in murine lymph nodes during homeostasis, dedifferentiation and inflammation.

74. Brulois, K. *et al.* A molecular map of murine lymph node blood vascular endothelium at single cell resolution. *Nat. Commun.* **11**, 3798 (2020).

This single cell RNA-sequencing study identifies non-high vein endothelial cells in murine lymph nodes which are important for myeloid cell migration into inflamed lymph nodes.

75. Goveia, J. *et al.* An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. *Cancer Cell* **37**, 21-36.e13 (2020).

This work describes endothelial heterogeneity at the single cell level in peritumoral lung tissues and lung tumor, and identifies endothelial cell subpopulations with immune cell recruiting, antigen presenting and scavenging capacity.

76. Qian, J. *et al.* A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. *Cell Res.* 1–18 (2020) doi:10.1038/s41422-020-0355-0.

77. Rohlenova, K. *et al.* Single-Cell RNA Sequencing Maps Endothelial Metabolic Plasticity in Pathological Angiogenesis. *Cell Metab.* **31**, 862-877.e14 (2020).

78. Allen, E. *et al.* Combined antiangiogenic and anti–PD-L1 therapy stimulates tumor immunity through HEV formation. *Sci. Transl. Med.* **9**, eaak9679 (2017).

79. He, B. *et al.* Remodeling of Metastatic Vasculature Reduces Lung Colonization and Sensitizes Overt Metastases to Immunotherapy. *Cell Rep.* **30**, 714-724.e5 (2020).

80. Johansson-Percival, A. *et al.* De novo induction of intratumoral lymphoid structures and vessel normalization enhances immunotherapy in resistant tumors. *Nat. Immunol.* **18**, 1207–1217 (2017).

81. Uhrig, A. *et al.* Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. *J. Leukoc. Biol.* **77**, 626–633 (2005).

82. Wu, J. *et al.* Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. *Immunology* **129**, 363–374 (2010).

83. Elvevold, K. *et al.* Liver sinusoidal endothelial cells depend on mannose receptor-mediated recruitment of lysosomal enzymes for normal degradation capacity. *Hepatology* **48**, 2007–2015 (2008).

84. Malovic, I. *et al.* The mannose receptor on murine liver sinusoidal endothelial cells is the main denatured collagen clearance receptor. *Hepatology* **45**, 1454–1461 (2007).

85. Diehl, L. *et al.* Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. *Hepatology* **47**, 296–305 (2008).

86. Limmer, A. *et al.* Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. *Eur. J. Immunol.* **35**, 2970–2981 (2005).

87. Limmer, A. *et al.* Efficient presentation of exogenous antigen by liver endothelial cells to CD8 + T cells results in antigen-specific T-cell tolerance. *Nat. Med.* **6**, 1348–1354 (2000).

88. Burgdorf, S., Kautz, A., Böhnert, V., Knolle, P. A. & Kurts, C. Distinct Pathways of Antigen Uptake and Intracellular Routing in CD4 and CD8 T Cell Activation. *Science* **316**, 612–616 (2007).

89. Carambia, A. *et al.* TGF-β-dependent induction of CD4+CD25+Foxp3+ Tregs by liver sinusoidal endothelial cells. *J. Hepatol.* **61**, 594–599 (2014).

90. Johansson, A. G., Lövdal, T., Magnusson, K., Berg, T. & Skogh, T. Liver cell uptake and degradation of soluble immunoglobulin G immune complexes in vivo and in vitro in rats. *Hepatology* **24**, 169–175 (1996).

91. Bertolino, P. *et al.* Early intrahepatic antigen-specific retention of naïve CD8+ T cells is predominantly ICAM-1/LFA-1 dependent in mice. *Hepatology* **42**, 1063–1071 (2005).

92. John, B. & Crispe, I. N. Passive and Active Mechanisms Trap Activated CD8 $^{+}$ T Cells in the Liver. *J. Immunol.* **172**, 5222–5229 (2004).

93. Shetty, S. *et al.* Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 Mediates the Transmigration of Regulatory T Cells across Human Hepatic Sinusoidal Endothelium. *J. Immunol.* **186**, 4147–4155 (2011).

94. Halpern, K. B. *et al.* Paired-cell sequencing enables spatial gene expression mapping of liver endothelial cells. *Nat. Biotechnol.* **36**, 962–970 (2018).

95. MacParland, S. A. *et al.* Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat. Commun.* **9**, 1–21 (2018).

This study provides a description of liver sinusoidal endothelial cells from different hepatic zones with distinct transcriptomes and putative immunomodulatory functions based on single cell RNA-sequencing data.

96. Gola, A. *et al.* Commensal-driven immune zonation of the liver promotes host defence. *Nature* 1–6 (2020) doi:10.1038/s41586-020-2977-2.

This paper describes how perivascular immune cells and liver endothelial cells cooperate for optimal host defense.

97. Berg, M. *et al.* Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells leads to tumor-specific CD8+ T cell tolerance. *Eur. J. Immunol.* **36**, 2960–2970 (2006).

98. Yu, X. *et al.* Immune modulation of liver sinusoidal endothelial cells by melittin nanoparticles suppresses liver metastasis. *Nat. Commun.* **10**, 1–14 (2019).

99. Sharma, A. *et al.* Onco-fetal Reprogramming of Endothelial Cells Drives Immunosuppressive Macrophages in Hepatocellular Carcinoma. *Cell* **183**, 377-394.e21 (2020).

This paper uses single cell RNA-sequencing to describe an immunosuppressive niche in hepatocellular carcinoma involving subtypes of endothelial cells, macrophages and regulatory T cells.

100. Knolle, P. A. *et al.* Role of sinusoidal endothelial cells of the liver in concanavalin A-induced hepatic injury in mice. *Hepatology* **24**, 824–829 (1996).

101. Xu, B. *et al.* Capillarization of Hepatic Sinusoid by Liver Endothelial Cell-Reactive Autoantibodies in Patients with Cirrhosis and Chronic Hepatitis. *Am. J. Pathol.* **163**, 1275–1289 (2003).

102. Ramachandran, P. *et al.* Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* **575**, 512–518 (2019).

The single cell RNA-sequencing data in this study allowed to identify a fibrosis-enriched endothelial cell subpopulation with distinct immunomodulatory characteristics.

103. Thiriot, A. *et al.* Differential DARC/ACKR1 expression distinguishes venular from non-venular endothelial cells in murine tissues. *BMC Biol.* **15**, (2017).

104. Girbl, T. *et al.* Distinct Compartmentalization of the Chemokines CXCL1 and CXCL2 and the Atypical Receptor ACKR1 Determine Discrete Stages of Neutrophil Diapedesis. *Immunity* **49**, 1062-1076.e6 (2018).

105. Nibbs, R. J. B. & Graham, G. J. Immune regulation by atypical chemokine receptors. *Nat. Rev. Immunol.* **13**, 815–829 (2013).

106. Hashimoto, S. *et al.* Postoperative Portal Hypertension Enhances Alloimmune Responses after Living-Donor Liver Transplantation in Patients and in a Mouse Model. *J. Immunol.* **203**, 1392–1403 (2019).

107. Almanzar, N. *et al.* A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature* **583**, 590–595 (2020).

108. Mammoto, A. & Mammoto, T. Vascular Niche in Lung Alveolar Development, Homeostasis, and Regeneration. *Front. Bioeng. Biotechnol.* **7**, (2019).

109. Jambusaria, A. *et al.* Endothelial heterogeneity across distinct vascular beds during homeostasis and inflammation. *eLife* **9**, e51413 (2020).

110. Kreisel, D. *et al.* Cutting Edge: MHC Class II Expression by Pulmonary Nonhematopoietic Cells Plays a Critical Role in Controlling Local Inflammatory Responses. *J. Immunol.* **185**, 3809–3813 (2010).

111. Travaglini, K. J. *et al.* A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature* **587**, 619–625 (2020).

112. Gillich, A. *et al.* Capillary cell-type specialization in the alveolus. *Nature* 1–5 (2020) doi:10.1038/s41586-020-2822-7.

This study identifies a subtype of alveolar capillaries with transcriptomic features suggesting antigen presentation capacity using single cell RNA-sequencing approaches.

113. Raredon, M. S. B. *et al.* Single-cell connectomic analysis of adult mammalian lungs. *Sci. Adv.* **5**, (2019).

114. Yang, J., Yan, J. & Liu, B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. *Front. Immunol.* **9**, (2018).

115. Lambrechts, D. *et al.* Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat. Med.* **24**, 1277–1289 (2018).

116. Klein, D. The Tumor Vascular Endothelium as Decision Maker in Cancer Therapy. *Front. Oncol.* **8**, 367 (2018).

117. Liu, S. *et al.* anlotinib alters tumor immune microenvironment by downregulating PD-L1 expression on vascular endothelial cells. *Cell Death Dis.* **11**, 1–16 (2020).

118. Claser, C. *et al.* Lung endothelial cell antigen cross-presentation to CD8 + T cells drives malaria-associated lung injury. *Nat. Commun.* **10**, 1–16 (2019).

119. Thacker, V. V. *et al. Rapid endothelial infection, endothelialitis and vascular damage characterise SARS-CoV-2 infection in a human lung-on-chip model*. http://biorxiv.org/lookup/doi/10.1101/2020.08.10.243220 (2020) doi:10.1101/2020.08.10.243220.

120. Angelidis, I. *et al.* An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. *Nat. Commun.* **10**, 963 (2019).

121. Meiners, S., Eickelberg, O. & Königshoff, M. Hallmarks of the ageing lung. *Eur. Respir. J.* **45**, 807–827 (2015).

122. Teuwen, L.-A., Geldhof, V., Pasut, A. & Carmeliet, P. COVID-19: the vasculature unleashed. *Nat. Rev. Immunol.* 1–3 (2020) doi:10.1038/s41577-020-0343-0.

123. Teijaro, J. R. *et al.* Endothelial Cells Are Central Orchestrators of Cytokine Amplification during Influenza Virus Infection. *Cell* **146**, 980–991 (2011).

124. Tundup, S. *et al.* Endothelial cell tropism is a determinant of H5N1 pathogenesis in mammalian species. *PLoS Pathog.* **13**, (2017).

125. Anderson, K. G. *et al.* Cutting Edge: Intravascular Staining Redefines Lung CD8 T Cell Responses. *J. Immunol.* **189**, 2702–2706 (2012).

126. Jourde-Chiche, N. *et al.* Endothelium structure and function in kidney health and disease. *Nat. Rev. Nephrol.* **15**, 87–108 (2019).

127. Molema, G. & Aird, W. C. Vascular Heterogeneity in the Kidney. *Semin. Nephrol.* **32**, 145–155 (2012).

128. Dmitrieva, N. I. & Burg, M. B. Elevated Sodium and Dehydration Stimulate Inflammatory Signaling in Endothelial Cells and Promote Atherosclerosis. *PLOS ONE* **10**, e0128870 (2015).

129. Wild, J. *et al.* Rubbing salt into wounded endothelium: Sodium potentiates proatherogenic effects of TNF-α under non-uniform shear stress. *Thromb. Haemost.* **112**, 183–195 (2014).

130. Dumas, S. J. *et al.* Single-Cell RNA Sequencing Reveals Renal Endothelium Heterogeneity and Metabolic Adaptation to Water Deprivation. *J. Am. Soc. Nephrol.* **31**, 118–138 (2020).

131. Rabelink, T. J. & de Zeeuw, D. The glycocalyx—linking albuminuria with renal and cardiovascular disease. *Nat. Rev. Nephrol.* **11**, 667–676 (2015).

132. Satchell, S. The role of the glomerular endothelium in albumin handling. *Nat. Rev. Nephrol.* **9**, 717–725 (2013).

133. Cross, A. R., Glotz, D. & Mooney, N. The Role of the Endothelium during Antibody-Mediated Rejection: From Victim to Accomplice. *Front. Immunol.* **9**, (2018).

134. Jane-wit, D. *et al.* Alloantibody and Complement Promote T Cell-Mediated Cardiac Allograft Vasculopathy through Non-Canonical NF-κB Signaling in Endothelial Cells. *Circulation* **128**, 2504–16 (2013).

135. Wu, H. *et al.* Single-Cell Transcriptomics of a Human Kidney Allograft Biopsy Specimen Defines a Diverse Inflammatory Response. *J. Am. Soc. Nephrol.* **29**, 2069–2080 (2018).

136. Pintavorn, P. & Ballermann, B. J. TGF-β and the endothelium during immune injury. *Kidney Int.* **51**, 1401–1412 (1997).

137. Su, H., Lei, C.-T. & Zhang, C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. *Front. Immunol.* **8**, (2017).

138. Bettelli, E. *et al.* Reciprocal developmental pathways for the generation of pathogenic effector T H 17 and regulatory T cells. *Nature* **441**, 235–238 (2006).

139. Lion, J. *et al.* Endothelial Cell Amplification of Regulatory T Cells Is Differentially Modified by Immunosuppressors and Intravenous Immunoglobulin. *Front. Immunol.* **8**, (2017).

140. Lion, J. *et al.* HLA Class II Antibody Activation of Endothelial Cells Promotes Th17 and Disrupts Regulatory T Lymphocyte Expansion. *Am. J. Transplant.* **16**, 1408–1420 (2016).

141. Taflin, C. *et al.* Human endothelial cells generate Th17 and regulatory T cells under inflammatory conditions. *Proc. Natl. Acad. Sci.* **108**, 2891–2896 (2011).

142. Young, M. D. *et al.* Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. *Science* **361**, 594–599 (2018).

143. Obermeier, B., Daneman, R. & Ransohoff, R. M. Development, maintenance and disruption of the blood-brain barrier. *Nat. Med.* **19**, 1584–1596 (2013).

144. Alvarez, J. I. *et al.* The Hedgehog Pathway Promotes Blood-Brain Barrier Integrity and CNS Immune Quiescence. *Science* **334**, 1727–1731 (2011).

145. Chen, M. B. *et al.* Brain Endothelial Cells Are Exquisite Sensors of Age-Related Circulatory Cues. *Cell Rep.* **30**, 4418-4432.e4 (2020).

146. Majerova, P. *et al.* Trafficking of immune cells across the blood-brain barrier is modulated by neurofibrillary pathology in tauopathies. *PLOS ONE* **14**, e0217216 (2019).

147. Ifergan, I. *et al.* The blood–brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. *Brain* **131**, 785–799 (2008).

148. Menard, C. *et al.* Social stress induces neurovascular pathology promoting depression. *Nat. Neurosci.* **20**, 1752–1760 (2017).

149. Grutzendler, J. *et al.* Angiophagy Prevents Early Embolus Washout But Recanalizes Microvessels Through Embolus Extravasation. *Sci. Transl. Med.* **6**, 226ra31-226ra31 (2014).

150. Howland, S. W., Gun, S. Y., Claser, C., Poh, C. M. & Rénia, L. Measuring antigen presentation in mouse brain endothelial cells ex vivo and in vitro. *Nat. Protoc.* **10**, 2016–2026 (2015).

151. Lopes Pinheiro, M. A. *et al.* Internalization and presentation of myelin antigens by the brain endothelium guides antigen-specific T cell migration. *eLife* **5**, (2016).

152. Yousef, H. *et al.* Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. *Nat. Med.* **25**, 988–1000 (2019).

153. Zhao, L. *et al.* Pharmacologically reversible zonation-dependent endothelial cell transcriptomic changes with neurodegenerative disease associations in the aged brain. *Nat. Commun.* **11**, 4413 (2020).

154. Ximerakis, M. *et al.* Single-cell transcriptomic profiling of the aging mouse brain. *Nat. Neurosci.* **22**, 1696–1708 (2019).

155. Janssens, R., Struyf, S. & Proost, P. The unique structural and functional features of CXCL12. *Cell. Mol. Immunol.* **15**, 299–311 (2018).

156. Brosseau, C., Colas, L., Magnan, A. & Brouard, S. CD9 Tetraspanin: A New Pathway for the Regulation of Inflammation? *Front. Immunol.* **9**, (2018).

157. Grubman, A. *et al.* A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat. Neurosci.* **22**, 2087–2097 (2019).

158. Marelli-Berg, F. M. *et al.* Cognate recognition of the endothelium induces HY-specific CD8+ T-lymphocyte transendothelial migration (diapedesis) in vivo. *Blood* **103**, 3111–3116 (2004).

159. Marelli-Berg, F. M., Frasca, L., Weng, L., Lombardi, G. & Lechler, R. I. Antigen Recognition Influences Transendothelial Migration of CD4+ T Cells. *J. Immunol.* **162**, 696–703 (1999).

160. Fu, H. *et al.* Self-recognition of the endothelium enables regulatory T-cell trafficking and defines the kinetics of immune regulation. *Nat. Commun.* **5**, (2014).

161. Garbuzova-Davis, S., Haller, E., Lin, R. & Borlongan, C. V. Intravenously Transplanted Human Bone Marrow Endothelial Progenitor Cells Engraft Within Brain Capillaries, Preserve Mitochondrial Morphology, and Display Pinocytotic Activity Towards BBB Repair in Ischemic Stroke Rats. *Stem Cells Dayt. Ohio* **35**, 1246–1258 (2017).

162. Lu, Y. *et al.* Increased acetylation of H3K14 in the genomic regions that encode trained immunity enzymes in lysophosphatidylcholine-activated human aortic endothelial cells – Novel qualification markers for chronic disease risk factors and conditional DAMPs. *Redox Biol.* **24**, 101221 (2019).

163. Li, X. *et al.* Anti-inflammatory cytokines IL-35 and IL-10 block atherogenic lysophosphatidylcholine-induced, mitochondrial ROS-mediated innate immune activation, but spare innate immune memory signature in endothelial cells. *Redox Biol.* **28**, (2019).

164. Drummer, C. *et al.* Trained Immunity and Reactivity of Macrophages and Endothelial Cells. *Arterioscler. Thromb. Vasc. Biol.* **41**, 1032–1046 (2020).

165. Stone, O. A. *et al.* Loss of pyruvate kinase M2 limits growth and triggers innate immune signaling in endothelial cells. *Nat. Commun.* **9**, 4077 (2018).

166. Huber, S. A. & Sartini, D. Roles of Tumor Necrosis Factor Alpha (TNF-α) and the p55 TNF Receptor in CD1d Induction and Coxsackievirus B3-Induced Myocarditis. *J. Virol.* **79**, 2659–2665 (2005).

167. Yang, L., Jhaveri, R., Huang, J., Qi, Y. & Diehl, A. M. Endoplasmic reticulum stress, hepatocyte CD1d and NKT cell abnormalities in murine fatty livers. *Lab. Invest.* **87**, 927–937 (2007).

168. Pandey, A. K., Brown, J. D., Harrison, D. G. & Itani, H. A. CD70 Modulates the Role of eNOS In Endothelial Cells. *FASEB J.* **32**, 845.7-845.7 (2018).

169. Watts, C., West, M. A. & Zaru, R. TLR signalling regulated antigen presentation in dendritic cells. *Curr. Opin. Immunol.* **22**, 124–130 (2010).

170. Brutkiewicz, R. R. Cell Signaling Pathways That Regulate Antigen Presentation. *J. Immunol.* **197**, 2971–2979 (2016).

171. Zhu, D. *et al.* Major histocompatibility complexes are up-regulated in glomerular endothelial cells via activation of c-Jun N-terminal kinase in 5/6 nephrectomy mice. *Br. J. Pharmacol.* **177**, 5131–5147 (2020).

172. Xie, R. *et al.* Phagocytosis by macrophages and endothelial cells inhibits procoagulant and fibrinolytic activity of acute promyelocytic leukemia cells. *Blood* **119**, 2325–2334 (2012).

173. Fauvarque, M. O. & Williams, M. J. Drosophila cellular immunity: a story of migration and adhesion. *J Cell Sci* **124**, 1373–82 (2011).

174. Thomas Graham, Tacke Robert, Hedrick Catherine C., & Hanna Richard N. Nonclassical Patrolling Monocyte Function in the Vasculature. *Arterioscler. Thromb. Vasc. Biol.* **35**, 1306–1316 (2015).

175. Almodovar, C. R. de, Luttun, A. & Carmeliet, P. An SDF-1 Trap for Myeloid Cells Stimulates Angiogenesis. *Cell* **124**, 18–21 (2006).

176. Schmidt, T. & Carmeliet, P. Bridges that guide and unite. *Nature* **465**, 697–699 (2010).

177. Burzyn, D., Benoist, C. & Mathis, D. Regulatory T cells in nonlymphoid tissues. *Nat. Immunol.* **14**, 1007–1013 (2013).

178. Davies, L. C., Jenkins, S. J., Allen, J. E. & Taylor, P. R. Tissue-resident macrophages. *Nat. Immunol.* **14**, 986–995 (2013).

179. Jenne, C. N. & Kubes, P. Immune surveillance by the liver. *Nat. Immunol.* **14**, 996–1006 (2013).

180. Han, X. *et al.* Construction of a human cell landscape at single-cell level. *Nature* 1–9 (2020) doi:10.1038/s41586-020-2157-4.

181. Daar, A. S., Fuggle, S. V., Fabre, J. W., Ting, A. & Morris, P. J. THE DETAILED DISTRIBUTION OF MHC CLASS II ANTIGENS IN NORMAL HUMAN ORGANS. *Transplantation* **38**, 293–298 (1984).

182. Mestas, J. & Hughes, C. C. W. Of Mice and Not Men: Differences between Mouse and Human Immunology. *J. Immunol.* **172**, 2731–2738 (2004).

183. Odobasic, D. *et al.* Glomerular Expression of CD80 and CD86 Is Required for Leukocyte Accumulation and Injury in Crescentic Glomerulonephritis. *J. Am. Soc. Nephrol.* **16**, 2012–2022 (2005).

184. Seino, K. *et al.* CD86 (B70/B7–2) on endothelial cells co-stimulates allogeneic CD4+T cells. *Int. Immunol.* **7**, 1331–1337 (1995).

185. Jollow, K. C., Zimring, J. C., Sundstrom, J. B. & Ansari, A. A. CD40 LIGATION INDUCED PHENOTYPIC AND FUNCTIONAL EXPRESSION OF CD80 BY HUMAN CARDIAC MICROVASCULAR ENDOTHELIAL CELLS1. *Transplantation* **68**, 430–439 (1999).

186. Prat, A., Biernacki, K., Becher, B. & Antel, J. P. B7 Expression and Antigen Presentation by Human Brain Endothelial Cells: Requirement for Proinflammatory Cytokines. *J. Neuropathol. Exp. Neurol.* **59**, 129–136 (2000).

187. Omari, K. I. M. & Dorovini-Zis, K. Expression and function of the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) in an in vitro model of the human blood–brain barrier. *J. Neuroimmunol.* **113**, 129–141 (2001).

188. Tan, P. H. *et al.* Phenotypic and functional differences between human saphenous vein (HSVEC) and umbilical vein (HUVEC) endothelial cells. *Atherosclerosis* **173**, 171–183 (2004).

189. Lozanoska-Ochser, B., Klein, N. J., Huang, G. C., Alvarez, R. A. & Peakman, M. Expression of CD86 on Human Islet Endothelial Cells Facilitates T Cell Adhesion and Migration. *J. Immunol.* **181**, 6109–6116 (2008).

190. Yao, S. *et al.* B7-H2 is a costimulatory ligand for CD28 in human. *Immunity* **34**, 729–740 (2011).

191. Aicher, A. *et al.* Characterization of Human Inducible Costimulator Ligand Expression and Function. *J. Immunol.* **164**, 4689–4696 (2000).

192. Khayyamian, S. *et al.* ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4+ T cells. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 6198–6203 (2002).

193. Shiao, S. L., McNiff, J. M. & Pober, J. S. Memory T Cells and Their Costimulators in Human Allograft Injury. *J. Immunol.* **175**, 4886–4896 (2005).

194. Hughes, C. C., Savage, C. O. & Pober, J. S. Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. *J. Exp. Med.* **171**, 1453–1467 (1990).

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AUTHOR INFORMATION: Correspondence should be addressed to P.C. (peter.carmeliet@kuleuven.be)

PEER REVIEW INFORMATION
NATURE REVIEWS IMMUNOLOGY THANKS RONEN ALON, XIAO-FENG YANG AND THE OTHER, ANONYMOUS, REVIEWER(S) FOR THEIR CONTRIBUTION TO THE PEER REVIEW OF THIS WORK.

TABLE 1: COMPARISON OF APC FEATURES IN PROFESSIONAL APCS (DCS, MACROPHAGES) AND ECS

Category	Specific feature	APC	Described in ECs	Refs
immunological synapse				
antigen presentation	MHC-I	yes	yes	29
	MHC-II	yes	yes	29
	CD1 (glycolipid antigens)	yes	yes, context-dependent[a]	35,166,167
	MR-1 (metabolite antigens)	yes	unknown / not examined	NA
	BTN3A1 (phosphoantigens)	yes	unknown / not examined	NA
co-stimulation	CD80, CD86, CD58, CD275, CD252, CD137L, CD154, CD70	yes	yes[b]	29,168
co-inhibition	PD-L1, PD-L2, CD155	yes	yes	29
cytokines	IL-1, IL-3, IL-5, IL-6, IL-8, IL-10, IL-11, G-CSF, GM-CSF, MCP-1, M-CSF, CCL5, TGF-beta, TNF	yes[c]	yes, polarization of immune cells by ECs during activation unknown	35
receptor/ signaling pathways				
extra / intracellular	TLR1-4, -6, -8, -9	yes	yes	169
intracellular	p38- / JAK/STAT- / JNK-signaling	yes	yes, relevance of p38 signaling unknown	29,170,171
antigen uptake / processing				
uptake	phagocytosis	yes	yes, population-dependent	13,150,172
processing	immunoproteasome	yes	yes, context-dependent	37,38
extracellular factors				
mechanical	shear stress	no	affects selectin and CAM expression and function, possibly tunes TCR signaling*	42,43,45–48,129
cell-cell interaction	interaction duration	long (several hours)	population-dependent?*	63,64

Although some of the features indicated in the table remain speculative and require further investigation, APC characteristics in professional APCs and ECs can overlap and differ in several manners.

[a]Not shown in human ECs. [b]CD80/CD86 not ubiquitously expressed by ECs, only *in vitro* in human ECs. [c]Polarization towards pro- / anti-inflammatory cytokine secretion. * indicates that these characteristics are speculative.

APC, antigen presenting cell; BTN3A1, Butyrophilin Subfamily 3 Member A1; CCL5, C-C Motif Chemokine Ligand 5; CD, cluster of differentiation; EC, endothelial cell; G-CSF, Granulocyte colony-stimulating factor; GM-CSF, Granulocyte-macrophage colony-stimulating factor JAK, Janus Kinase; JNK, c-Jun N-terminal Kinase; M-CSF, Macrophage colony-stimulating factor; MCP-1, Monocyte chemoattractant protein-1; MHC, major histocompatibility complex; MR-1, Major Histocompatibility Complex, Class I-Related; NA, not applicable; PD-L1, programmed death ligand-1; STAT, signal transducer and activator of transcription; TGF-β, Transforming growth factor beta 1; TLR, Toll-Like Receptor; TNF, Tumor Necrosis Factor.

FIGURE LEGENDS

FIGURE 1: IMMUNOMODULATION BY ECS IN LYMPH NODES AND LIVER

Known and putative insights into immunomodulation by endothelial cells (ECs) in lymph nodes and liver. **a** | Lymph nodes (LNs) 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 . **b** | During inflammation (indicated by the red background), HEVs (upper panel) and venuous (bottom panel) ECs in LNs can recruit various immune cells, such as neutrophils, monocytes and effector T (T_{eff}) 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, antibody-mediated anti-angiogenic therapy (AAT) or delivery of LIGHT protein , combined with immune checkpoint blockade (ICB), was found to increase HEV biogenesis, thereby promoting tumor immunity and immunotherapy[78-80]. **d** | Interestingly, activated HEVs express additional immunomodulatory genes, which may impair dendritic cell activation (via reverse CD137-CD137L signaling)[69], alter macrophage differentiation (via macrophage migration inhibitory factor (MIF)[70,71]), or inhibit T cell activation (via thrombospondin-1 (TSP-1)[72]).

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 PD-L1 upon cross-presentation of gut flora-derived antigens via MHC class I, or through the induction of T_{reg} 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** | Periportal 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 signaling, thereby altering macrophage phenotypes in a context-dependent manner. **h** | In hepatocellular carcinoma, malignant hepatocyte -derived VEGF induces $PLVAP^+$

tumor ECs (TECs) to form an immunosuppressive niche of FOLR2$^+$ macrophages and T_{reg} cells. Therapeutic approaches that break LSEC-mediated immune tolerance can impair liver metastasis in preclinical models of metastatic melanoma, breast and colon carcinoma[98]. **i** | In regions of liver fibrosis, ACKR1$^+$ ECs might recruit and modulate/polarize macrophages through the secretion of differentiation factors such as protein GAS6 in a contextual manner. Asterisks (*) indicate recent insights which we considered novel for IMEC biology.

ACKR1, atypical chemokine receptor 1; DC, dendritic cell; EC, endothelial cell; FOLR2, folate receptor beta; GAS6, growth arrest – specific 6; HEV, high endothelial venule; LSEC, liver sinusoidal endothelial cell; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; PD-L1, programmed death ligand 1; PLVAP; plasmalemma vesicle associated protein; TEC, tumor endothelial cell; Tn cell, naive T cell; Treg; regulatory T cell; Tsp-1, thrombospondin-1; VEGF, vascular endothelial growth factor.

FIGURE 2: EC IMMUNOMODULATION IN LUNG, KIDNEY AND BRAIN

Known and putative insights on EC immunomodulation per tissue type. **a** | In lung cancer, tumor ECs (TECs) are generally immunosuppressive since they display decreased expression levels of antigen presentation molecules, ICAM1 and various cytokines and chemokines compared to normal lung ECs. Further immunosuppressive features of lung TECs include the elevated expression of FAS-L, which induces CD8$^+$ T cell apoptosis, and high levels of inhibitory molecules such as PD-L1. In contrast, chronic tumor inflammation (indicated by a red background) induces pro-inflammatory HEV-like ECs, which can also occur in other tissues with chronic inflammation. **b** | In malaria infection, specific lung immunomodulary ECs (IMECs) take up and present parasite antigen to CD8$^+$ T cells, which then kill ECs by cytolysis, leading to vascular leakage and lung damage. **c** | Lung IMECs in alveoli are involved in immune cell

recruitment and in controlling a delicate balance between immunity and tolerance to pathogens through high expression of MHC class II (MHC-II). This possibly involves VEGF, which has immunosuppressive function. However, the exact underlying mechanisms require further investigation. **d** | Glomerular ECs with a particularly thick glycocalyx (as depicted, though other ECs generally also have a glycocalyx which is not shown) impair immune cell infiltration by shielding adhesion/selectin molecules (here represented by CAM, which include mainly but not exclusively integrin ligands) on their surface (therefore not visible in the figure). In kidney disease (indicated by red background), glycocalyx shedding exposes these molecules and promotes immune cell recruitment and inflammation. **e** | Glomerular ECs clear immune complexes through uptake from the circulation and transcellular transport into the glomeruli for subsequent removal by resident macrophages. **f** | MHC-I and -II expressing renal IMECs are a target of donor-specific antibodies (DSA) after kidney transplantation, leading to context-dependent EC activation and altered immunomodulation. **g** | Renal ECs are phenotypically heterogeneous, due to their exposure to a heterogeneous microenvironment of differing osmolarities, affecting their inflammatory status. The exact underlying mechanisms and consequences, depending on their anatomical location, require further investigation *in vivo.* **h** | The healthy brain is an immune privileged site, and BBB-ECs contribute to this by having tight intercellular junctions and with low or absent expression of adhesion molecules. Upon EC activation in disease (indicated by red background), the BBB is breached and the brain parenchyma is no longer immune privileged. **i** | ECs from the aged mouse brain show heterogeneity in (increased) cytokine signaling in arteries, veins and capillaries, possibly increasing immune cell recruiting properties and consequently increasing EC immune modulatory status and reducing immune privilege. Asterisks (*) indicate recent insights which we considered novel for IMEC biology. BBB, blood-brain-barrier, CAM, cell adhesion molecule; DSA, donor specific antibodies; EC, endothelial cell; HEV, high endothelial venule, HLA, human leukocyte antigen; ICAM1, intercellular

adhesion molecule; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; mOsm, milliosmoles; PD-L1, programmed death ligand-1; TEC, tumor endothelial cell; TGF, transforming growth factor; TLR, toll-like receptor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

BOX 1: EVOLUTIONARY ORIGIN OF ECS

From an evolutionary perspective, it is not surprising that certain subtypes of ECs have immunomodulatory properties. Indeed, in non-vertebrates, blood vessels initially consisted only of hollow matrix tubes, with motile hemocytes that patrolled the body for immune surveillance[173]. Later in evolution as vertebrates developed, these hemocytes diverged to become adherent ECs or immune cells, such as patrolling monocytes that scan the vascular lining for cellular debris[174], neutrophils, or tissue resident immune cells such as macrophages [173,175,176]. Given that tissue resident immune cells such as macrophages also have tissue-specific characteristics[177–179], one might speculate that immune cells and ECs have co-evolved to allow optimal tissue immune homeostasis.

BOX 2: SPECIES-SPECIFIC DIFFERENCES IN IMEC FEATURES

Different kinds of immune modulatory ECs (IMECs) have been described in humans, mice and other mammals such as rats, but interspecies differences exist and these have been best described in humans and mice. For example, microvascular ECs in most human tissues constitutively express HLA-II *in vivo*[11,180,181], but mouse ECs only express MHCII under inflammatory conditions[182]. Moreover, cross-presentation (exogenous antigen presentation to $CD8^+$ T cells) occurs in mouse ECs[36,86,97,118,150] but has

not (yet) been documented in human ECs. Importantly, co-stimulatory molecules can differ between species. CD80 and CD86 are expressed by mouse ECs in a context-dependent manner[23,110,183], but not consistently detected in human ECs, and have so far only been observed *in vitro*[29,184–189]. In humans, but not mice, inducible costimulatory-ligand (ICOS-L) binds to CD28[190] albeit at a different binding site than CD80/ CD86[191], and insufficiently for naïve T cell activation[192]. Interestingly, CD58 (the most potent co-stimulatory molecule of memory T cells in human ECs[193,194]) has a ~50-fold higher affinity for its receptor CD2 than for its mouse counterpart CD48, suggesting interspecies differences for memory T cell activation by ECs[11]. Lastly, as a deficiency in cytokine receptors, such as IL7-receptor, differentially impacts immunity in humans *versus* mice[182], the immunomodulatory effect of EC-derived cytokines might also be species-dependent.

TOC:

IN THIS PERSPECTIVE, CARMELIET AND COLLEAGUES DISCUSS EVIDENCE FOR SPECIFIC IMMUNOMODULATORY ROLES FOR ENDOTHELIAL CELLS, AND HOW THESE CELLS MAY BE TARGETED FOR IMMUNOTHERAPY.