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HIGHLIGHTS

Basic

REVIEW

Sensing context: Inhibitory receptors on non-hematopoietic cells

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Similar to immune cells, non-hematopoietic cells recognize microbial and endogenous threats. Their response to these stimuli is dependent on the environmental context. For example, intact intestinal epithelium expresses pattern recognition receptors (PRRs) but should tolerate commensal bacteria, while damaged epithelium should respond promptly to initiate an immune response. This indicates that non-hematopoietic cells possess mechanisms to sense environmental context and regulate their responses. Inhibitory receptors provide context sensing to immune cells. For instance, they raise the threshold for activation to prevent overzealous immune activation to harmless stimuli. Inhibitory receptors are typically studied on hematopoietic cells, but several of these receptors are expressed on non-hematopoietic cells. Here, we review evidence for the regulation of non-hematopoietic cells by inhibitory receptors, focusing on epithelial and endothelial cells. We explain that inhibitory receptors on these cells can sense a wide range of signals, including cell-cell adhesion, cell-matrix adhesion, and apoptotic cells. More importantly, they regulate various functions on these cells, including immune activation, proliferation, and migration. In conclusion, we propose that inhibitory receptors provide context to non-hematopoietic cells by fine tuning their response to endogenous or microbial stimuli. These findings prompt to investigate the functions of inhibitory receptors on non-hematopoietic cells more systematically.

Keywords: endothelium · epithelium · homeostasis · inhibitory receptors · non-hematopoietic cells

Introduction

Similar to immune cells, non-hematopoietic cells express pattern recognition receptors and can be in contact with microbial and endogenous patterns [1–4]. Their response to these stimuli depends on the situation. For example, exposure of epithelial cells to a certain microbe can be harmless when the epithelial barrier is intact, whereas the same microbe can be dangerous when the barrier is breached. In the latter case, epithelial cells should become activated and produce inflammatory cytokines to recruit immune cells, which in turn kill microbes at the wound interface and contribute to tissue repair [5]. Thus, non-hematopoietic cells need to sense the context in which they receive a microbial or endogenous stimulus.

We previously argued that inhibitory receptors can provide context to immune cells by acting as negative feedback receptors or as threshold receptors. Negative feedback receptors are upregulated after activation to terminate the immune response, whereas threshold receptors are expressed on non-activated cells and provide a threshold to prevent unnecessary immune activation, for instance, in response to harmless stimuli [6]. We also recently reviewed that multiple inhibitory receptors recognize endogenous and microbial patterns that can indicate danger, homeostasis, or both [7]. As such, these inhibitory pattern recognition receptors (iPRRs) can form a regulatory counterpart to activating PRRs.

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We proposed that regulation by iPRRs may occur mostly in tissues that require a high activation threshold, such as tissues that face continuous microbial exposure (e.g., barrier tissues such as the intestine and skin) or tissues that have low tolerance for immunopathology (e.g., the brain, heart, or eyes) [7]. Mechanistically, inhibitory receptors usually contain an ITIM, or in some cases an immunoreceptor tyrosine-based switch motif (ITSM), which becomes phosphorylated upon receptor ligation. This leads to the recruitment of Src homology-2 (SH2) domain-containing inhibitory effectors such as SH2 domain-containing phosphatases (SHP)-1, SHP-2, SH2 domain-containing inositol 5'phosphatase (SHIP), or C-terminal Src kinase (Csk), which in turn inhibit the signaling of activating receptors [8, 9].

Even though inhibitory receptors are almost exclusively studied in hematopoietic cells, several of these receptors are expressed on non-hematopoietic cells. In this review, we examine the evidence for the regulation of non-hematopoietic cells by ITIM-bearing inhibitory receptors. Herein, we focus on the regulation of epithelial cells and endothelial cells, for two reasons. First, these cells are located in barrier tissues and thus provide an example of cells that may particularly benefit from regulation by inhibitory receptors. Second, studies which have addressed ITIM-dependent signaling on non-hematopoietic cells are sufficiently available for epithelial cells and endothelial cells, while only limited for other cell types. Since some inhibitory receptors have multiple functions, we only regard ITIM-dependent signaling as an inhibitory receptor function.

Inhibitory receptors on epithelial cells

Epithelial cells express iPRRs

The immune function of epithelial cells comprises two major tasks: i) maintaining tissue integrity to prevent microbial invasion, which requires cell adhesion, proliferation, and migration, and ii) forming the first line of defense of the immune system by secreting inflammatory mediators. All of these functions could potentially be controlled by inhibitory receptors. Indeed, epithelial cells express several inhibitory receptors. They widely express CEACAM1 (also known as CD66a) and PVR (also known as Necl-5 or CD155) [10, 11], while CD300LF expression is restricted to tuft cells, a rare secretory epithelial cell with immune-related functions [12]. All three receptors contain one or more intracellular ITIMs and are known to inhibit the immune functions of hematopoietic cells, although PVR has been primarily studied as a ligand for other immune receptors [13–15]. Of note, these are inhibitory receptors of which expression on epithelial cells has been described in the literature. However, RNA sequencing databases such as protein atlas report that some epithelial cell types may express more inhibitory receptors, albeit at lower levels than in immune cells [16]. This requires further validation on protein level. In addition, the human genome encodes many uncharacterized genes potentially encoding for ITIM-bearing receptors, which could also be expressed on epithelial cells [9].

CEACAM1, PVR, and CD300LF are pleiotropic receptors, each responding to multiple endogenous and microbial patterns ([17, 18] and reviewed in [7]). CEACAM1 binds itself, other CEACAMs, and microbial Ig-fold proteins. PVR binds the adhesion molecule Nectin-3, the matrix protein vitronectin, the immune receptors TIGIT, CD96, and DNAM-1, and forms the entry receptor for poliovirus. CD300LF binds phosphatidylserine (PS) and phosphatidylethanolamine (PE) on apoptotic cells and forms the entry receptor for murine norovirus (but not for human norovirus). This ligand repertoire classifies these receptors as iPRRs [7] in agreement with a regulatory role in barrier tissues that are highly exposed to microbial patterns.

Inhibitory receptors on epithelial cells regulate proliferation, immune activation, and migration

Which functions do these inhibitory receptors regulate on epithelial cells? Firstly, CEACAM1 and PVR both contribute to cell-cell adhesion by interacting in trans with respectively CEACAM1 or Nectin-3 on neighboring epithelial cells [19, 20]. In addition, PVR can mediate cell-matrix contact by binding to vitronectin [17]. For PVR it has not been addressed whether the ITIM is required for cell adhesion, but cell adhesion mediated by CEACAM1 is ITIM-independent [21]. However, *trans* homophilic interaction between CEACAM1 on neighboring cells does induce CEACAM1 ITIM phosphorylation and SHP-2 recruitment [22]. This has multiple potential functional consequences. First, CEACAM1 is already known for 25 years to inhibit proliferation of epithelial cells [21, 23, 24]. This involves ITIM-mediated signaling: CEACAM1 overexpression in human lung epithelial cells inhibits cell growth in confluent cell layers, whereas cells with overexpression of CEACAM1 in which the tyrosines of both ITIMs have been mutated to phenylalanine to abrogate signaling (Y459F/Y486F) continue to proliferate and overgrow [25]. Second, CEACAM1 signaling may affect cell migration, although contradicting findings are reported: CEACAM1 overexpression inhibits migration of MC38 colon epithelial cells in an ITIM-dependent manner [26], but others have found that CEACAM1 overexpression enhances migration of HT-29 and Caco-2 colon epithelial cells [27, 28]. Third, CEACAM1 can dampen immune activation of epithelial cells, as it inhibits TLR2-induced IL-8 production by airway epithelial cells in response to its endogenous ligand CEACAM8 [29] and its microbial ligands from *Moraxella catarrhalis* and *Neisseria meningitides* [30]. Using HEK293T cells, inhibition of the *M. catarrhalis*-induced TLR2 response was shown to require Y459 but not Y486 [30]. Notably, the requirement of ITIM-mediated signaling to the function of these receptors is not always specifically addressed, and thus requires further examination. For example, the interaction between CEACAM1 and HopQ from *Helicobacter pylori* has been shown to induce ITIM phosphorylation and enhanced IL-8 release [31], but it was not addressed whether ITIM-mediated signaling is responsible for this immune-activating effect.

For PVR and CD300LF, ITIM activation and the functional consequence thereof has to our knowledge not been addressed in epithelial cells. However, studies using fibroblast cell lines with receptor overexpression indicate that these receptors have the capacity for ITIM-mediated signaling in non-hematopoietic cells and may provide hints toward their function on epithelial cells. PVR ligation inhibits fibroblast adhesion to fibronectin while it enhances cell migration, which is abolished by ITIM mutation or co-expression of a dominant negative SHP-2 mutant [32]. In line with this, several other studies show that PVR enhances fibroblast migration, albeit without addressing the requirement of ITIM phosphorylation (reviewed in ref. [33]). In addition, PVR has been shown to enhance fibroblast proliferation, which is reversed upon cell-cell contact-induced endocytosis of PVR [34], posing a different potential mechanism by which an inhibitory receptor may prevent cell overgrowth. However, again the involvement of ITIM signaling was not addressed. CD300LF overexpression in fibroblasts positively regulates phagocytosis of apoptotic cells by recognition of PS [35]. Phagocytosis increases even further after Y→F mutation of Y241, Y289, or Y325, which are the central tyrosines of two ITIMs and an ITSM motif, respectively. In contrast, phagocytosis decreases after mutation of Y276 which is present in a binding site for the PI3K subunit p85α [35]. Together, this indicates that CD300LF can simultaneously transmit inhibitory signals via its ITIMs and activating signals via its p85α-binding motif.

In summary, inhibitory receptors may regulate various processes on epithelial cells, including proliferation, immune activation, and migration. However, more studies are needed to specifically address the requirement of ITIM signaling of inhibitory receptors on epithelial cells.

Splice isoform expression affects function and localization of inhibitory receptors

One factor that may cause variable outcomes of inhibitory receptor ligation is the differential expression of isoforms that do or do not contain intracellular signaling motifs, as a result of alternative splicing. PVRα and CEACAM1-L isoforms contain a long intracellular tail with ITIMs, as opposed to PVRδ and CEACAM1-S isoforms with a short intracellular tail and no ITIMs (reviewed in [36, 37]). CEACAM1-S not only lacks ITIM-mediated signaling, but also interferes with signaling of CEACAM1-L by disrupting the formation of CEACAM1-L-*cis* dimers, which leads to decreased recruitment of SHP-2 [22]. Interestingly, differential expression of CEACAM1-L/S isoforms may regulate the function of CEACAM1 in different contexts. For example, a low L:S ratio is found in subconfluent, proliferating cells, whereas a high L:S ratio is found in confluent rat epithelial cells [38]. Thus, predominant CEACAM1-L expression may inhibit proliferation in confluent epithelium, while predominant CEACAM1-S expression may counteract the growth-inhibitory effect of CEACAM1-L in proliferating, sub-confluent epithelial layers.

Additionally, the ITIMs of CEACAM1 and PVR act as sorting signal in polarized epithelial cells. CEACAM-S is only localized on the apical surface of epithelial cells, whereas CEACAM-L is localized on the apical and lateral surface [39]. For this lateral sorting, Y515 but not Y488 is needed [40]. PVRδ is expressed on the apical and basolateral surface of polarized epithelial cells, whereas PVRα is only localized on the basolateral surface, which depends on interaction between the tyrosine in its ITIM motif and the mu1B subunit of the clathrin adaptor complex [41]. The localization of ITIM-containing isoforms may indicate that these inhibitory receptors preferentially respond to basolateral ligation such as contact with neighboring cells. It remains to be determined whether this also leads to preferential suppression of basolateral activating signals, e.g., due to the limited molecular reach of phosphatases such as SHP-1 [42], or whether basolateral inhibitory receptors can also suppress signals received at the apical side. Importantly, some activating PRRs such as TLR3 and TLR5 are also preferentially expressed on the basolateral side of epithelial cells [43, 44]. This may indicate that tissue damage and basolateral pathogen invasion can activate epithelial cells with a double kick: by increased stimulation of activating PRRs, while concurrently releasing the break of inhibitory PRRs.

Additional factors can affect inhibitory receptor function on epithelial cells. For example, CEACAM1 function can be controlled by proteolytic cleavage of its ectodomain and intracellular domain [45, 46], and by altered expression of CEACAM5 and CEACAM6 [25]. Taken together, inhibitory receptor function is controlled by a complex interplay of splice isoform expression, subcellular localization, ligand expression, and posttranslational modifications.

Inhibitory receptors provide context to epithelial cells

In conclusion, epithelial cells express inhibitory receptors through which they can sense a wide range of signals which give information on their context, including cell-cell adhesion, cell-matrix adhesion, apoptotic cells, and presence of microbes (Fig. 1). Moreover, these receptors have the potential to inhibit cellular processes in epithelial cells such as proliferation and immune activation, as has been shown for CEACAM1. Based on these findings, we propose that inhibitory receptors on epithelial cells can fine tune their responses to external events depending on the context. For example, interaction between CEACAM1 molecules on adjacent cells may indicate that the epithelial barrier is intact, thereby signalling that proliferation or an immune response is not needed. In other words, CEACAM1 may signal a context of safety.

In contrast, pathogens such as *M. catarrhalis* and *N. meningitidis* may exploit this by ligating CEACAM1 to evade immune activation. Similarly, poliovirus and norovirus may benefit from ITIM-mediated immune inhibition via respectively PVR and CD300LF. Pathogens that bind CEACAM1 have also been shown to inhibit exfoliation of infected epithelial cells, by enhancing epithelial cell binding [47, 48]. However, this effect is shared by other CEACAMs which do not contain an ITIM, and is therefore most likely mediated by the adhesive properties of CEACAMs rather than ITIM

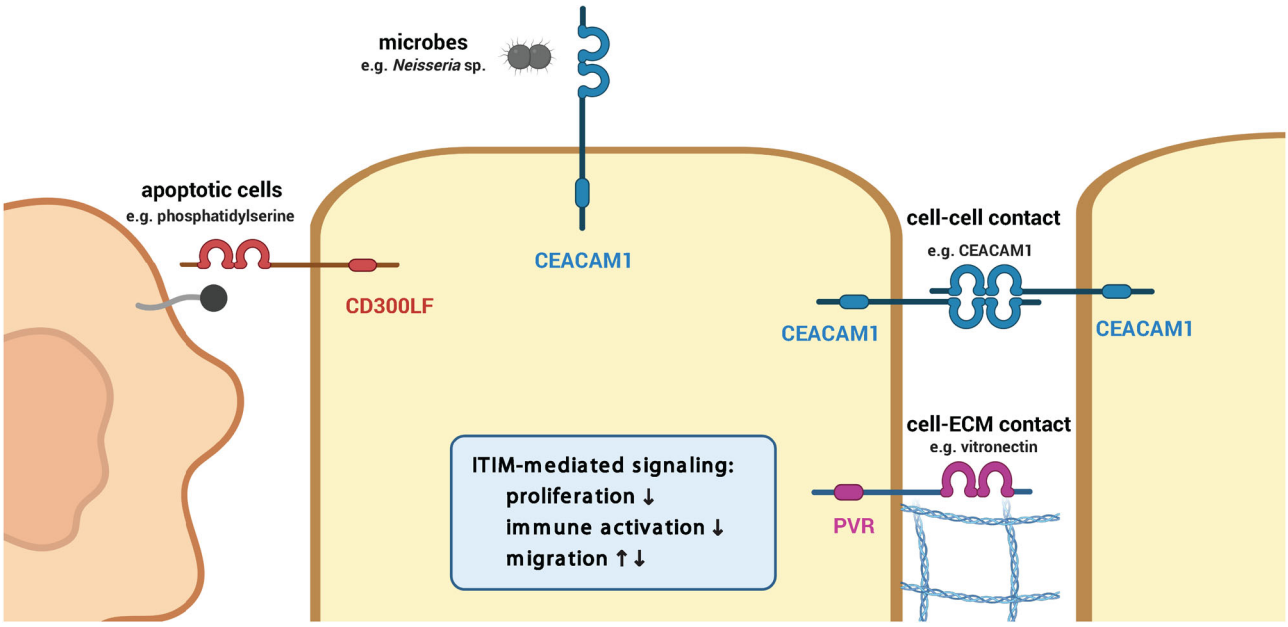

Figure 1. Inhibitory receptors on epithelial cells can sense several signals such as cell–cell contact (e.g., CEACAM1 with CEACAM1 or other CEACAMs), cell–matrix contact (e.g., PVR–vitronectin), apoptotic cells (e.g., CD300LF–PS/PE), or presence of microbes (e.g., CEACAM1–*Neisseria* species). These interactions potentially control proliferation, immune activation, and migration. The figure was created with biorender.com.

signaling. Notably, CEACAM1 also binds Opa adhesins expressed by commensal Neisseriae species, although the functional consequence of this interaction has not been addressed [49]. It would be interesting to investigate if such interactions could also be of benefit to the host, by dampening immune activation to microbes in locations in which an immune response would do more harm than good.

Inhibitory receptors on endothelial cells

Endothelial cells express inhibitory receptors that recognize cell–cell contact

Just like epithelial cells, endothelial cells have context-dependent immune functions. For instance, they need to maintain vascular integrity, while also allowing leukocyte transmigration during inflammation. Endothelial cells can be exposed to microbial patterns during infection, and to a wide variety of endogenous stimuli such as DAMPs and cytokines [50]. Thus, endothelial cells require regulation to ensure appropriate responses to these stimuli, indicating a potential role for inhibitory receptors.

Endothelial cells express several inhibitory receptors, of which PECAM-1 (also known as CD31) is a well-known marker for endothelium [51]. In addition, they express CEACAM1 [52], PVR [53], and, in some tissues, SIRPα (also known as SHPS-1) [54, 55]. SIRPα and PECAM-1 have extensively been studied for their immune inhibitory function on hematopoietic cells [56, 57]. As in epithelial cells, these receptors may signal tissue integrity. All of them interact *in trans* with ligands expressed on neighboring cells, namely CEACAM1 with CEACAM1, PECAM-1 with PECAM-1, PVR with Nectin-3, and SIRPα with CD47 [57–59]. In addition, their ligands are expressed on leukocytes, indicating potential interaction with transmigrating leukocytes. Lastly, PECAM-1 has been shown to act as a mechanosensor, as it becomes rapidly ITIM phosphorylated and recruits SHP-2 upon fluid sheer stress and direct mechanical pressure [60], although this was not found in primary human endothelial cells *ex vivo* [61]. What is the consequence of these interactions?

Inhibitory receptors on endothelial cells regulate endothelial cell migration and leukocyte diapedesis

PECAM-1 resembles CEACAM1 in its function, as *trans* homophilic PECAM-1 and CEACAM1 interactions both contribute to endothelial cell-cell adhesion and vascular integrity (reviewed in [62, 63]). For PECAM-1 this has been shown to be ITIM-independent [64]. Conversely, *trans* homophilic PECAM-1 interaction may induce ITIM phosphorylation, as PECAM-1 becomes phosphorylated after binding to immobilized PECAM-1 [65]. This has several potential functional outcomes. Firstly, several studies show that PECAM-1 enhances endothelial cell migration (reviewed in [51]), although mixed results are found on whether ITIM signaling positively or negatively contributes to this. Some studies report increased PECAM-1 ITIM phosphorylation in confluent cell cultures and ITIM-mediated *inhibition* of cell migration [66, 67], whereas others show the exact inverse, namely increased ITIM phosphorylation in wounded cell cultures and ITIM-mediated *enhancement* of cell migration [68, 69]. Of note,

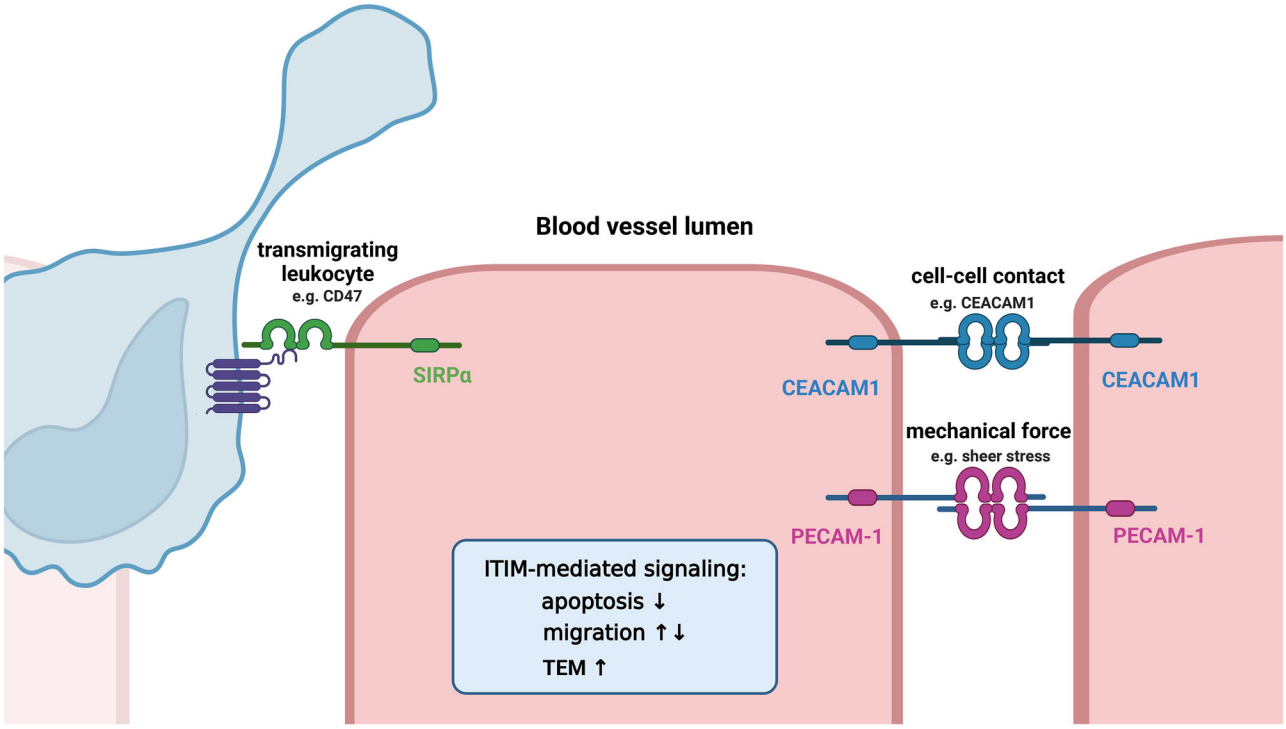

Figure 2. Inhibitory receptors on endothelial cells can sense contact with neighboring endothelial cells, transmigrating leukocytes or mechanical force. These interactions potentially control apoptosis, migration, and leukocyte transendothelial migration. The figure was created with biorender.com.

the latter studies used PECAM-1 transfected REN mesothelial cells as a substitute for endothelial cells. In support for pro-migratory effects of inhibitory receptor signaling on endothelial cells, CEACAM1 increases endothelial cell migration in an Y488-dependent manner [70]. Similarly, SIRPα increases the migration of melanoma and CHO cells via its ITIMs, suggesting it may have a similar function on endothelial cells [71]. Thus arguably the most consistent finding is that inhibitory receptor signaling enhances endothelial cell migration. Mechanistically, this has been explained by SHP-2-mediated RhoA regulation, although controversy exists on whether SHP-2 activates [72] or inhibits [73] RhoA, and de-phosphorylation of focal adhesion components such as paxillin, which in turn increases the turnover of focal adhesions [68, 69]. Still, increased PECAM-1 tyrosine phosphorylation in wounded cell cultures seems counterintuitive, as one may expect that wounding induces loss of cell-cell contact and thereby *decreased trans* homophilic PECAM-1 ligation. Interestingly though, wounding-induced PECAM-1 ITIM phosphorylation occurs independent of homophilic binding [74], indicating a different ligand for PECAM-1 in this setting.

Second, endothelial cell-expressed PECAM-1, PVR, and SIRPα all have been shown to facilitate leukocyte transendothelial migration (TEM) upon interaction with their leukocyte-expressed ligands [55, 75–77]. Mechanistically, this was suggested to involve targeting of PECAM-1 toward the membrane engulfing the translocating leukocyte, which required Y663 but not SHP-2 recruitment [78]. However, two recent independent studies confirm that PECAM-1 and SIRPα mediate TEM in an ITIM- and SHP-2-dependent manner [55, 79]. Remarkably, for PECAM-1 this seems to require its inactivation rather than activation, as contact with leukocytes *decreases* PECAM-1 ITIM phosphorylation and SHP-2 recruitment, while SHP-2 is targeted to VE-cadherin instead [79]. As a functional consequence thereof, VE-cadherin is internalized, leading to the loosening of endothelial junctions [55, 79]. Possibly, these different proposed mechanisms represent distinct steps in how inhibitory receptors mediate TEM. For example, the ITIM may first serve as a sorting signal to target the inhibitory receptor to the membrane engulfing the leukocyte, where it becomes phosphorylated to recruit SHP-2, after which it becomes dephosphorylated to transfer SHP-2 to VE-cadherin. In support, PECAM-1 is itself a substrate of SHP-2 [68].

Inhibitory receptor function may again be affected by differential expression of splice isoforms. In addition to its full-length form with two ITIMs, PECAM-1 contains isoforms that lack one or both ITIMs. In human endothelial cells, the full-length form of PECAM1 is predominantly expressed, while murine endothelial cells abundantly express the Δ14,15 isoform which lacks one ITIM (reviewed in [80]). In contrast, SIRPα does not express an ITIM-less isoform but instead an isoform that lacks a large part of the extracellular domain, which may affect its ligation [81].

In summary, inhibitory receptor signaling on endothelial cells has the potential to regulate endothelial cell migration and TEM (Fig. 2). Studies on the underlying mechanism are partially conflicting and may be resolved by further investigations that include

ITIM mutants, SHP-2 mutants and the monitoring of ITIM phosphorylation across several time points, in addition to controlling factors that may influence inhibitory receptor signaling such as cell density and expression of splice isoforms.

Inhibitory receptors on endothelial cells may protect endothelial integrity

Another special feature of the endothelium is its resistance to cell death, as it needs to withstand high concentrations of inflammatory mediators during inflammation while maintaining its integrity. This becomes evident in patients with allograft rejection, where host effector T cells damage the donor organ while the donor capillary endothelium remains relatively unharmed [82]. Remarkably, PECAM-1 is sufficient to confer resistance against TNF and cytotoxic T lymphocytes to vascular endothelium, which requires both of its ITIMs and correlates with SHP-2 recruitment and Erk/Akt pathway activation [83]. In addition, both PECAM-1 and PVR inhibit apoptosis induced by serum starvation [65, 84, 85]. Together with the ability of inhibitory receptors to enhance endothelial cell migration, these findings suggest that inhibitory receptors on endothelial cells may protect endothelial integrity, not only by acting as adhesion molecules, but also by inhibiting apoptosis and by enhancing migration in an ITIM-dependent manner. This may be of particular relevance during inflammation or wounding, where endothelial integrity is challenged. In support, mice with endothelial cell-specific PECAM-1 deficiency only have a mild phenotype under homeostatic conditions, but show exaggerated inflammation and vascular permeability in inflammatory disease models, although it remains to be determined whether this phenotype is caused by a lack of ITIM signaling ([86] and reviewed in [57]). Perhaps surprising in this regard is the finding that inhibitory receptors mediate TEM, as this is considered a pro-inflammatory function. How and why these functions concur needs to be a topic of future investigation.

Inhibitory receptors on non-hematopoietic cells: Role in disease

In line with the regulation of cell growth and migration by inhibitory receptors, several non-hematopoietic malignancies show aberrant inhibitory receptor expression, including CEACAM1 and PVR (reviewed in [87, 88]). Whether expression is preferentially up- or down-regulated may depend on the dominant function of the particular receptor in that setting. For example, CEACAM1 expression is abolished in several epithelial malignancies in line with its growth-suppressive effects, whereas in other epithelial malignancies, CEACAM1 expression is linked to metastatic spread, which may be related to its ability to enhance cell migration [88]. Altered expression may also include aberrant isoform expression; malignant cells of non-small cell lung carcinomas patients express predominantly CEACAM-S, whereas healthy-appearing lung epithelial cells of the same patients express predominantly CEACAM1-L [89]. Some non-hematopoietic malignancies even express the inhibitory receptor PD1 [90], which is usually exclusively expressed by immune cells. Importantly, these findings indicate that inhibitory receptor blockade in cancer immunotherapy may have side effects on non-hematopoietic cells expressing the targeted receptor, which may concern healthy tissue and/or the non-hematopoietic malignancy itself. Indeed, PD1 blockade has been shown to affect tumor-cell intrinsic PD1 signaling, albeit with the dual outcome, with studies indicating enhanced lung carcinoma growth [91] but decreased melanoma growth [92] as a result of tumor-cell intrinsic PD1 blockage.

Not only malignancies but also pathogens can exploit inhibitory receptors on non-hematopoietic cells, such as binding of pathogenic *Neisseria* species to CEACAM1, poliovirus to PVR, and murine norovirus to CD300LF. Similarly, *Clostridium perfringens* and *Streptococcus pneumoniae* have been shown to target PECAM-1 to bind to the endothelium and invade underlying tissue [93, 94].

To further understand the role of inhibitory receptors in non-hematopoietic malignancies and infection biology, future studies should differentiate between ITIM-dependent and -independent functions. For example, PVR is also implicated in malignancies due to its function as a ligand of TIGIT on NK cells and T cells, where it inhibits cytotoxic activity towards cancer cells and thereby promotes tumor growth [87]. Similarly, it is not always addressed to what extent pathogens use inhibitory receptors for adhesion or also to benefit from ITIM-mediated signaling.

Inhibitory receptors on non-hematopoietic cells in expensive tissues

We focused this review on non-hematopoietic cells that are present in barrier tissues—an environment that is characterized by its high exposure to microbes and other exogenous stimuli. As we previously argued, cells in this environment benefit from a high activation threshold to prevent unnecessary immune activation [7]. A high activation threshold may also be beneficial in so-called expensive tissues such as the heart, brain, and eyes, which are characterized by a low regenerative capacity and therefore also a low tolerance to immunopathology [7]. Notably, non-hematopoietic cells in these tissues do express PRRs. For example, neurons express TLR2 and TLR4, which contribute to ischemia-induced neuronal cell death [95]. Therefore, to prevent overzealous PRR signaling, "expensive" cells may also be regulated by inhibitory receptors. In support, neurons widely express PD1 and SIRPα (reviewed by [96, 97]), and PD1 ligation in neurons inhibits neuronal excitability and pain via SHP-1 [98]. SIRPα and PD1 expression is also found on neurons in the retina [99, 100]. Likewise, SIRPα is expressed on human cardiomyocytes [101, 102] and protects against cardiac hypertrophy via inhibition of TLR4 signaling [103]. In summary, inhibitory receptors may regulate non-hematopoietic cells in various tissues. This may occur especially in cells or tissues that benefit from a high activation threshold, such as expensive tissues.

Discussion and future perspective

Here, we reviewed the evidence for the regulation of non-hematopoietic cells by inhibitory receptors. Based on the described findings, we propose that inhibitory receptors not only provide context to immune cells but also to non-hematopoietic cells. For example, inhibitory receptors on epithelial cells can sense cell–cell contact and thereby signal that an immune response or proliferation is not needed. In contrast, on endothelial cells, sensing of cell wounding by inhibitory receptors may stimulate cell migration to re-establish barrier integrity, indicating cell-specific functions of inhibitory receptors. Seemingly counter-intuitive, some of the described inhibitory receptor functions are activating rather than inhibitory, but it should be kept in mind that negative regulation of an inhibitory process leads to a positive outcome. For example, inhibition of cell adhesion leads to the enhancement of cell motility and migration. Likewise, at a signaling level, SHP-2 causes activation of ras/ERK/MAPK pathway by dephosphorylating negative regulators of this pathway (reviewed by [104]).

Taken together, there is a clear need to investigate the functions of inhibitory receptors on non-hematopoietic cells more specifically and systematically. Not only because of their potential involvement in disease, but also because some of these receptors are (potential) therapeutic targets as immune checkpoints, such as CEACAM1 and PVR [105–107], which may affect non-hematopoietic cells expressing the same receptor. Importantly, many of the described studies have been done using cancer cell lines, and thus need to be repeated *in vivo*, in primary cells *in vitro* (whenever possible), or in intermediate models such as organoids. Experiments with inhibitory receptors with mutated ITIMs and/or mutants of downstream phosphatases will help clarify the downstream signaling. Lastly, more than sixty inhibitory receptors have been functionally characterized, but over 300 putative ITIM-bearing receptors are encoded in the human genome [9]. This raises the possibility that non-hematopoietic cells are regulated by several additional inhibitory receptors that help them to respond appropriately to their environment.

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Abbreviations: **Csk**: C-terminal Src kinase · **ITSM**: immunoreceptor tyrosine-based switch motif · **PRR**: pattern recognition receptor · **PS**: phosphatidylserine · **SH2**: Src homology-2 · **SHP**: SH2 domain-containing phosphatase

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