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DEL-1-regulated immune plasticity and inflammatory disorders

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Abstract

In contrast to traditional immune cell-centered viewpoints, recent studies suggest that tissues are not passive recipients of immunity but have a ‘regulatory say’ over the host inflammatory response. Identification of tissue-derived homeostatic molecules regulating immune plasticity is seminal for understanding the inherent regulatory potential of different organs on the immune response. DEL-1 is a secreted multi-domain protein interacting with integrins and phospholipids and regulates, depending on its expression location, distinct stages of the host inflammatory response (from myelopoiesis over leukocyte recruitment to efferocytosis and resolution of inflammation). Here we synthesize recent evidence of DEL-1 as an exemplar local regulatory factor in the context of tissue immune plasticity and inflammatory disorders (such as, periodontitis, multiple sclerosis, and pulmonary disorders), and discuss its potential as a therapeutic agent.

Keywords

Inflammation; homeostasis; periodontitis; myelopoiesis; resolution; integrins; DEL-1; *EDIL3*

Local tissue signals and functional immune plasticity

The maintenance or restoration of host tissue **homeostasis** (see Glossary) necessitates that the function of immune cells be tailored to the requirements and changing conditions of the tissue where they are recruited to or reside. This adaptation, often referred to as functional **immune plasticity**, results from an intimate crosstalk with the tissue microenvironment [1,2]. The dissection of local regulatory mechanisms underlying functional immune plasticity has been considered critical for gleaning insights into immune-driven inflammatory diseases and cancer [1,3,4]. For instance, under the influence of microenvironmental signals, tumor-associated macrophages or neutrophils may become polarized in a manner that fosters tumor progression and suppresses adaptive immunity [5].

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In a similar context, it has been proposed that local tissues are not passive recipients of immune surveillance but have a ‘regulatory say’ over the initiation and regulation of immune and inflammatory responses [1,3,4]. Specifically, homeostatic factors derived from a specific tissue can regulate initiation and termination of the immune response by fine-tuning the recruitment and activation of immune cells as well as the functional plasticity of both tissue-resident and recruited immune cells. To this end, local tissues communicate with and instruct the immune cells through stromal and parenchymal cell-derived signals, including growth factors, cytokines, antimicrobial peptides, and other locally acting homeostatic factors, as well as by intercellular adhesive interactions [3,4,6]. It can be reasoned that the location of homeostatic molecules within the tissue microenvironment is critical, as compartmentalized expression can facilitate optimal performance with cell type-specific actions and spatial regulation of the immune response.

Multi-functional proteins associating with the extracellular matrix upon their secretion from specific cellular components may regulate immune plasticity in a tissue-specific context. This is because such molecules, depending on their cellular origin, may become localized in certain locations of the tissue (‘geographic restriction’) and are limited to interact with specific cell types using a subset or all of their functional domains (depending on the availability of receptors or ligands on the interactive partner). This geographic restriction may enable spatiotemporal regulation of cell plasticity in a manner tailored to the needs of the tissue in question. For instance, osteopontin and milk fat globule-EGF factor 8 (MFG-E8) are integrin-binding multifunctional proteins that contribute to homeostasis in specific tissues (*e.g.*, as a component of collagenous extracellular matrix of mineralized tissues, osteopontin modulates osteoclast differentiation [7] and MFG-E8 expressed by certain cell types, such as endothelial cells, myofibroblasts or dendritic cells may exert functions promoting tissue plasticity [8–10]).

These emerging concepts, which are crucial for understanding tissue immunity in health and disease, are strongly supported and exemplified by studies on the biology and functional propensities of an endogenous molecule, secreted from distinct tissue-resident cells and associating with the cell surface or the extracellular matrix, the developmental endothelial locus-1 (DEL-1) [11–13]. DEL-1 is a versatile, multi-domain protein that can interact with phospholipids and distinct integrins thereby regulating the host inflammatory response at different stages (ranging from the production of neutrophils in the bone marrow to their recruitment to inflamed tissues and their eventual clearance by macrophages when neutrophils undergo **apoptosis** during resolution of inflammation; Fig. 1). As a consequence of its many non-redundant functions, low expression or deficiency of DEL-1 in mice and humans has been associated with inflammatory disorders, especially pathologies driven by interleukin (IL)-17 (*e.g.*, multiple sclerosis and inflammatory bone loss) [13–16].

In this review, we synthesize the evidence that DEL-1 is a major local endogenous determinant of context-dependent functional immune plasticity tailored to maintain or restore tissue homeostasis. Studies on DEL-1 have provided experimental support for previously mostly theorized concepts, such as the aforementioned tissue-based control of immunity and the importance of compartmentalized expression of homeostatic factors [13,14,17–21]. As DEL-1 expression is higher in select tissues (*e.g.*, brain and lungs [12]), it

is likely that similar functions in tissues that express little or no DEL-1 are performed by other regulators, yet to be discovered. DEL-1 may therefore serve as a paradigm to better understand how local tissues regulate immune cells and thus determine health vs. disease.

Structure and interactions of DEL-1

DEL-1 is a 52-kDa protein consisting of three *N*-terminal epidermal growth factor (EGF)-like repeats (E1–E3) and two *C*-terminal discoidin I-like domains (C1–C2). DEL-1 is therefore also designated as EGF-like repeats and discoidin I-like domains 3 (EDIL3) [22,23]. Whereas ‘DEL-1’ is predominantly used in the literature for referring to the protein, ‘*EDIL3*’ is invariably used to denote the DEL-1-encoding gene [11,13,14,16–20,23–27]. DEL-1 interacts with α_v integrins via an RGD motif in its second EGF repeat [22,24,26], whereas it binds to glycosaminoglycans and phosphatidylserine (PS) via its discoidin I-like domains [11,13,21]. DEL-1 can also bind to β_2 integrins, heterodimeric molecules with a distinct CD11 subunit and a common CD18 subunit [14,15,25,28]. CD11a/CD18 (LFA-1, lymphocyte function-associated antigen 1; $\alpha_L\beta_2$ integrin) is a crucial player in leukocyte recruitment to an infected or inflamed tissue by mediating the firm adhesion of leukocytes to the vascular endothelium, and contributing to the transmigration of leukocytes through the vessel wall [29]. CD11b/CD18, also known as Mac-1 ($\alpha_M\beta_2$ integrin), mediates intraluminal crawling of leukocytes on the endothelium, an activity during which leukocytes search for an appropriate site for transmigration [29].

The aforementioned interactions mediate important DEL-1 functions: DEL-1 can capture platelet microparticles via PS and mediate microparticle clearance by endothelial cells in a manner that involves $\alpha_v\beta_3$ integrin on the latter [18]. Moreover, the molecular DEL-1 bridge between PS on apoptotic cells and $\alpha_v\beta_3$ integrin on macrophages mediates the **efferocytosis** of apoptotic cells by the phagocytes [13,21]. The ability of DEL-1 to bind glycosaminoglycans enables its deposition to the extracellular matrix [11], where it can be sequestered and concentrated for potential functional interactions (*e.g.*, with immune cells). Moreover, by binding to β_2 integrins on leukocytes, DEL-1 can prevent the functional interactions of LFA-1 with counter-receptors on vascular endothelial cells, such as the intercellular adhesion molecule-1 (ICAM-1), as well as the Mac-1-dependent leukocyte functions [14,15,25,28,30] (Fig. 1, **middle inset**).

Thus, DEL-1 interacts with distinct integrins and with PS, properties that enable DEL-1 to mediate important immune regulatory functions that impact innate leukocytes, predominantly neutrophils, throughout their entire lifespan (from their production to their eventual clearance). These regulatory functions are discussed below.

Regulation of myelopoiesis

Peripheral infectious or inflammatory triggers stimulate myeloid cell production (myelopoiesis) in the bone marrow (BM), which is critical for the replenishment of peripheral neutrophils consumed during infection or inflammation; this process involving a feedback loop between periphery and the BM is termed **emergency myelopoiesis** [31–33]. Emergency myelopoiesis represents an important homeostatic mechanism in immunity and

inflammation. Consistent with its homeostatic roles, DEL-1 was shown to contribute to emergency myelopoiesis. Specifically DEL-1 was found to be a constituent of the **hematopoietic stem cell (HSC) niche** [20]. The niche supports HSC survival and renewal, and promotes maintenance of HSC quiescence, which prevents HSC DNA damage and replicative senescence [34]. Distinct cellular players of the BM niche, namely, arteriolar endothelial cells, cells of the osteoblastic lineage and a specialized perivascular mesenchymal stromal cell type, the so-called CXCL12-abundant reticular (CAR) cells, produce DEL-1 [20].

DEL-1 in the niche was shown to interact with the $\beta 3$ integrin on HSCs and promote proliferation of long-term (LT)-HSCs and their differentiation toward myeloid cells [20] (Fig. 1, **left inset**). DEL-1-mediated enhanced myelopoiesis occurs under steady-state but predominantly under stress conditions, such as after hematopoietic cell transplantation or LPS-induced systemic inflammation or granulocyte-colony stimulating factor (G-CSF)-induced emergency myelopoiesis. In this regard, myeloid cell replenishment following the BM response to G-CSF administration or LPS-induced systemic inflammation is defective in DEL-1-deficient mice owing to reduced hematopoietic stem and progenitor cell proliferation [20]. In other words, the critical homeostatic myelopoietic response of the BM under stress conditions fails in the absence of DEL-1. Although DEL-1 expression in the lung or cultured endothelial cells is inhibited by inflammatory stimuli [25,35], the expression of DEL-1 in the BM is not significantly altered by LPS-induced systemic inflammation [20]. Therefore, DEL-1 may persist at sufficient levels to effectively regulate myelopoiesis. In this regard, the expression of DEL-1 in the CAR cells of the BM is higher than its expression in BM endothelium [20] and DEL-1 expression in CAR cells might be differentially regulated by inflammatory stimuli as compared to its regulation in endothelial cells. In contrast to DEL-1, osteopontin restrains emergency myelopoiesis in the BM through cell type-specific regulation of apoptosis; intracellular osteopontin promotes the apoptosis of myeloid progenitors, whereas secreted osteopontin inhibits the apoptosis of differentiated lymphoid cells [36]. As opposed to DEL-1 deficiency, endothelial cell type-specific overexpression of DEL-1 in the perivascular niche increases BM cellularity, associated with expansion of functional LT-HSCs with enhanced potential for myeloid-lineage reconstitution [37].

Although the above-discussed studies were performed in mouse models, it should be noted that most fundamental knowledge regarding the function of human stem cells and their niche was derived from relevant studies in mouse models [34,38–40]. In addition, human bone marrow mesenchymal stromal cells display high expression of DEL-1 [20]. Thus, the ability of DEL-1 to control steady-state and stress-related myelopoiesis in the mouse BM could be relevant in the human system and hence harnessed therapeutically (see Potential DEL-1-based therapeutic applications). The homeostatic effects of DEL-1 on myeloid cells is not restricted to their production but also regulates their functions in peripheral tissues as outlined below.

Regulation of neutrophil recruitment and inflammation

As alluded to above, endothelial cell-derived DEL-1 binds to neutrophil LFA-1 integrin and suppresses their adhesion to ICAM-1 on vascular endothelial cells, thereby restraining the extravasation of neutrophils and initiation of inflammation [25,29,41,42]. Being a regulator of neutrophil recruitment, DEL-1 could, at least in principle, modulate local inflammation and disease in different settings.

Periodontal tissue

The concept that DEL-1 could act as a local endogenous factor to prevent chronic inflammation was first demonstrated in the setting of the oral inflammatory disease periodontitis (Box 1) [14]. A major portion of the inflammatory tissue destruction in human or experimental mouse periodontitis is attributed to supernumerary neutrophils that can cause collateral damage by releasing inflammatory mediators, reactive oxygen species, and connective tissue-degrading enzymes [43–46]. DEL-1-deficient (*Edil3*^{-/-}) mice were shown to spontaneously develop heavy neutrophil infiltration in the gingival tissue and pronounced loss of the underlying bone at young age, which (young age) is not normally associated with pathological inflammation and bone loss [14]. The destructive inflammation due to DEL-1 deficiency was not only mediated by excessive LFA-1-dependent neutrophil recruitment, but also required IL-17 receptor signaling. Indeed, mice doubly deficient in DEL-1 and the IL-17 receptor were protected from developing periodontitis, in contrast to mice singly deficient in DEL-1 that also exhibited excessive production of IL-17 [14].

These observations led to mechanistic studies, which revealed that DEL-1 and IL-17 not only mediate opposite functions but are also reciprocally regulated (Fig. 2): IL-17 directly suppresses endothelial DEL-1 expression and thereby promotes LFA-1-dependent neutrophil recruitment, whereas DEL-1 counteracts IL-17 production and IL-17-dependent inflammation [14,35]. Mechanistically, IL-17 decreases DEL-1 expression in a glycogen synthase kinase 3 β (GSK3 β)-dependent process, which inhibits the binding of the critical transcription factor CCAAT/enhancer-binding protein β (C/EBP β) to the *EDIL3* promoter, thereby downregulating *EDIL3* (DEL-1) transcription [35] (Fig. 3). Consistent with these experimental findings, independent groups observed an inverse relation between the expression of DEL-1 and IL-17 in human gingival biopsy samples, with DEL-1 levels being higher in healthy gingiva and IL-17 levels in inflamed gingiva [14,47]. The inhibition of DEL-1 by IL-17 is an additional mechanism whereby IL-17 can stimulate neutrophil recruitment to peripheral tissues. Additional established mechanisms include the ability of IL-17 to orchestrate granulopoiesis and neutrophil mobilization and chemotaxis through the induction of G-CSF and CXC chemokines [48]. On the other hand, the exact mechanism, by which DEL-1 inhibits IL-17 is not clarified yet. The reciprocal regulation between DEL-1 and IL-17 keeps the protective and detrimental effects of leukocyte recruitment in balance (Fig. 2).

Intriguingly, DEL-1 expression progressively declines with aging. Gingival DEL-1 expression is severely diminished in old mice (> 18 months of age), correlating with exuberant neutrophil recruitment and IL-17 expression, resulting in IL-17-dependent inflammatory bone loss [14,49] (Fig. 2). It therefore appears that genetic DEL-1 deficiency

confers an accelerated aging phenotype in young mice. Periodontal inflammation in old mice could be reversed by replenishing local levels of DEL-1. Indeed, intra-gingival injection of recombinant DEL-1 (expressed as an Fc-fusion protein; DEL-1-Fc) was shown to inhibit LFA-1-dependent neutrophil infiltration and IL-17 production [14]. An aging-related decline in gingival DEL-1 expression was recently confirmed in humans by an independent group [47]. Given that DEL-1 is downregulated by inflammatory stimuli such as IL-17 and TNF [25,35], the aging-related DEL-1 reduction might be a consequence of “inflamm-aging”, the increased chronic inflammatory status often associated with old age [50–52]. On the other hand, periodontitis is considered to be a contributory factor to inflamm-aging [53,54]. Therefore, the aging-related DEL-1 deficiency might be a factor contributing to inflamm-aging by aggravating periodontitis in old age. Overall, inflamm-aging could be both a cause and a consequence in a feed-forward loop where inflammation and chronic disease reinforce each other.

Central nervous system

Interestingly, the brain displays highly abundant expression of DEL-1 well above the levels seen in the gingiva or the lungs [14,25]. This observation implies a priority for protection against inflammation, which is consistent with its being an immune-privileged organ. Indeed, DEL-1 is an integral component of the immune privilege of the central nervous system [15]. Similar to periodontitis, DEL-1 deficiency in experimental autoimmune encephalomyelitis (EAE; murine model of **multiple sclerosis**) resulted in enhanced IL-17 production and neutrophil infiltration in the spinal cord, accompanied by increased tissue destruction (demyelination and axonal loss) [15]. Moreover, DEL-1-deficient mice displayed greater blood-brain barrier disruption than DEL-1 proficient mice in the course of EAE. IL-17 can promote disruption of the blood-brain-barrier and increase endothelial permeability [55,56]; hence the upregulation of IL-17 in DEL-1 deficiency provides a potential mechanism for the disrupted barrier in DEL-1-deficient mice. Moreover, the increased neuroinflammation due to DEL-1 deficiency was associated with enhanced neutrophil infiltration to the central nervous system, which further supports the increasingly important role of these innate immune cells in EAE and multiple sclerosis pathogenesis and the function of neutrophils as effector cells of IL-17-dependent inflammation [57,58]. Both disease severity and neutrophil accumulation seen in DEL-1-deficient mice were abrogated in mice with concomitant deficiency in DEL-1 and IL-17 receptor [15]. Hence, the IL-17–neutrophil inflammatory axis promotes enhanced EAE pathogenesis in DEL-1 deficiency. Importantly, DEL-1 expression was decreased in chronic-active, but not in chronic-inactive, multiple sclerosis lesions in humans [15], implying that the pathogenesis of the human disease might in part be driven (or exacerbated) by deficiency or low levels of DEL-1. The notion that reduced DEL-1 expression may predispose to multiple sclerosis is consistent with *EDIL3* being a susceptibility gene in human multiple sclerosis [23].

Lungs and other peripheral tissues

Genetic deficiency of DEL-1 was associated with acute lung neutrophilic inflammation [25] and bleomycin-induced lung fibrosis in mouse models [19], whereas treatment of mice with mesenchymal stem cells engineered to overexpress DEL-1 inhibited LPS-induced lung injury and decreased neutrophil numbers in bronchoalveolar lavage [59]. Using

bronchoalveolar lavage fluid (BALF) from patients with allergic asthma and healthy controls, a study demonstrated an inverse relationship between the levels of DEL-1 and IL-17, with DEL-1 dominating in health and IL-17 in allergic asthma [16]. Treatment of BALF cells with DEL-1 inhibited the expression of IL-17 and eosinophil-associated cytokines, suggesting that DEL-1 might play a protective role in allergic asthma [16]. Mice with endothelial cell-specific overexpression of DEL-1 were protected (as compared to wild-type controls) against acute blood-mediated inflammatory reactions associated with pancreatic islet transplantation to the liver [30]. Genetic DEL-1 deficiency in mice exacerbated adrenal gland dysfunction associated with systemic inflammation as compared to wild-type controls [60]. Moreover, studies in humans with or without Sjogren's syndrome, as well as in a relevant mouse model, associated decreased DEL-1 expression with salivary gland inflammation, including a marked increase in IL-17 [61].

Together, these studies suggest that DEL-1 acts as a local gatekeeper of leukocyte recruitment and (predominantly IL-17-driven) inflammation in different disease settings. However, the anti-inflammatory actions of DEL-1 may not be solely linked to its property to inhibit the initiation of inflammation, as DEL-1 was more recently shown to contribute to the resolution of inflammation, as discussed below.

Regulation of efferocytosis and inflammation resolution

The induction of acute inflammation with rapid neutrophil recruitment in response to infection or tissue injury is vital for host protection. The pro-inflammatory cytokine IL-17 promotes neutrophil recruitment not only by inducing neutrophil-specific chemokines but also by inhibiting endothelial DEL-1 expression, thereby swiftly enhancing tissue inflammation [14,35,48]. Of equal importance, however, is the ability of the host to resolve the inflammatory response in a timely manner, which allows restoration of tissue integrity and homeostasis. Defective inflammation resolution (Box 2) underlies the pathogenesis and is the common denominator of many chronic autoimmune and inflammatory diseases, including periodontitis [62–64].

Resolution of periodontal inflammation

Potential involvement of DEL-1 in resolution was initially suggested by findings that the aforementioned ability of IL-17 to downregulate DEL-1 is reversed by specific pro-resolving mediators, such as resolvin-D1 (RvD1). Specifically, RvD1 induces phosphatidylinositol 3-kinase (PI3K)/Akt signaling, which inhibits GSK3 β downstream of IL-17 signaling, thereby suppressing its inhibitory effect on C/EBP β , a critical transcription factor required for *EDIL3* expression [35] (Fig. 3). This finding suggested that DEL-1 expression might resurge during resolution, which could be partially attributed to the rising levels of resolvins. Indeed, DEL-1 protein concentrations were significantly elevated in the **gingival crevicular fluid** of periodontitis patients upon treatment (mechanical debridement to remove the pathogenic biofilm), which led to clinical inflammatory resolution [13,65]. Similarly, DEL-1 mRNA and protein expression is also upregulated during the resolution of murine periodontal inflammation [13]. The model used involved **ligature-induced periodontitis** for 5 days, at which time the ligature was removed to abrogate the pathogenic microbial challenge and

initiate resolution. In this model, resurgence of DEL-1 expression in the course of inflammation resolution was associated with upregulation of TGF β 1 and TGF β 2 protein and mRNA, while expression of the proinflammatory cytokines IL-6 and IL-17 was downregulated, compared to the levels of these cytokines in the inductive phase of periodontitis [13]. This DEL-1 upregulation during periodontitis resolution is functionally important, as DEL-1-deficient mice exhibited non-resolving inflammation and persistent bone loss, even when the ligatures were removed [13]. Therefore, DEL-1 may not only regulate the initiation of periodontal inflammation but might also participate in its resolution. This concept may also apply to other inflammatory diseases, given that the reciprocal relationship between DEL-1 and IL-17, first described in the context of periodontitis, was shown to be relevant also in diseases affecting different tissues [15,16,61].

Efferocytosis and macrophage reprogramming

The pro-resolving function of DEL-1 was also demonstrated in acute monosodium urate crystal-induced peritoneal inflammation (a model resembling the self-limited inflammation of human gout) and was attributed to the ability of DEL-1 to effectively promote efferocytosis. In this regard, DEL-1 binds with high affinity via its C-terminal discoidin-like domains to the 'eat-me' signal PS on apoptotic cells and interacts via its N-terminal RGD motif with the macrophage α v β 3 integrin; these features enable DEL-1 to act as a bona fide efferocytic 'bridge' to promote the uptake of apoptotic cells (Fig. 4). DEL-1 has about 50% amino-acid sequence identity with and is structurally similar to MFG-E8, which was earlier shown to promote efferocytosis through the same mechanism [66]. In this regard, defective efferocytosis of apoptotic B cells in germinal centers due to MFG-E8 deficiency leads to autoimmune pathology [67]. Interestingly, osteopontin inhibits MFG-E8-mediated efferocytosis of apoptotic cells in germinal centers [68], although it is currently uncertain whether osteopontin is expressed at appropriate locations to also affect Del-1-mediated efferocytosis and inflammation resolution. To date, the biological functions of Del-1 and MFG-E8 have not been sufficiently compared in side-by-side experiments. However, given that these molecules are expressed by different cell subsets, or in different tissues, and are regulated by distinct transcription factors [13,21,35,69], one may expect that the two molecules often mediate non-redundant roles.

Importantly, the DEL-1-encoding *Edil3* gene is among the top upregulated genes in macrophages undergoing efferocytosis [70], thus suggesting a continuous expression of DEL-1 for as long as the efferocytosis lasts during the resolution phase. The pro-efferocytic function of DEL-1 promoted reprogramming of macrophages to a pro-resolving phenotype, in a manner that involved the activity of liver-X-receptor [13], indicating that DEL-1 is an endogenous regulator of functional immune plasticity tailored to restore tissue homeostasis. Intriguingly, defective efferocytosis in DEL-1-deficient mice resulted in decreased levels of the resolvins RvD1 and RvE1, thus further impairing resolution. However, even when exogenous RvD1 was administered, its pro-resolving effect in experimental periodontitis and peritoneal inflammation largely failed in DEL-1-deficient (albeit not in wild-type) mice [13]. Therefore, DEL-1 is a non-redundant effector that mediates resolution of inflammation and promotes production of resolvins, thereby generating a positive feedback loop that reinforces inflammation resolution (Fig. 4).

Location principle

DEL-1 tends to associate with the cell surface or the extracellular matrix in the proximity of DEL-1-secreting cells, hence, its diffusion is likely limited [11,12]. Therefore, the expression of DEL-1 at a site where it is specifically needed for action might be a crucial regulatory issue for immune plasticity and homeostatic immunity. This notion was tested using different transgenic mice with cell type-specific overexpression of DEL-1. The results of these experiments attributed the anti-leukocyte recruitment action to endothelial cell-derived DEL-1, while the efferocytic/pro-resolving function was linked to macrophage-derived DEL-1 [13]. In keeping with the homeostatic state and responsibilities of resident peritoneal macrophages [71], DEL-1 was identified as a specific gene of these cells. Specifically, DEL-1 is predominantly expressed by GATA6⁺ resident peritoneal macrophages [21,72]. Whether resident macrophages of other tissues also express DEL-1 and engage it in their homeostatic functions is not clear at this point. These findings reveal a novel location-dependent homeostatic principle (**'location principle'**) in the spatial regulation of the immune response (Fig. 4). In other words, the compartmentalized expression allows DEL-1 (and perhaps other regulatory secreted molecules) to exert differential functions important for tissue homeostasis in an appropriate context. In conclusion, endothelial-derived DEL-1 primarily inhibits inflammation initiation (leukocyte recruitment), whereas macrophage-derived DEL-1 predominantly promotes the resolution of inflammation (by enhancing efferocytosis) [13].

Interestingly, a similar location-dependent principle was recently demonstrated to regulate the actions of two highly homologous chemokines CXCL1 and CXCL2 that interact with the CXCR2 receptor [73]. Specifically, the differential roles for these chemokines were governed by their distinct cellular sources. While CXCL1 derived from TNF-stimulated endothelial cells and pericytes, CXCL2 was produced mainly by neutrophils [73], in line with earlier observations that these closely related chemokines are differentially regulated [74]. Consistent with their compartmentalization, CXCL1 promotes neutrophil crawling on endothelial cells and the subendothelium, while CXCL2 mediates neutrophil transmigration through endothelial junctions. The latter action of CXCL2 required the atypical chemokine receptor 1, which is enriched within endothelial junctions, acting as a junctional chemokine "depot" for CXCL2 [73].

Together, these findings lend further support to the importance of compartmentalized expression of immune regulators within a tissue and to the concept that local tissues are not passive recipients of immunity but can proactively regulate the induction and resolution of the host inflammatory response [1,3,4]. Molecules performing such important homeostatic functions could become targets for novel therapies as outlined below for DEL-1.

Potential DEL-1-based therapeutic applications

Periodontitis

The property of DEL-1 to not only interfere with the initiation of periodontal inflammation but also to promote its resolution suggested that this homeostatic molecule could be harnessed therapeutically in the context of human periodontitis. A proof-of-concept study

tested the therapeutic potential of DEL-1 in ligature-induced inflammatory bone loss in non-human primates (NHP). NHP and human periodontitis share key clinical, histological and microbiological features [75]. Ligature-induced periodontitis was performed in the posterior maxillary teeth of NHPs (young adult cynomolgus monkeys), which were treated locally with DEL-1 (expressed as a fusion protein with the Fc fragment of human IgG; DEL-1-Fc), using a split-mouth experimental design: DEL-1-Fc was administered to the gingiva on the one side of the maxilla, and the contralateral side received Fc control; thus, each animal served as its own control. Treatments were initiated three days after ligature placement and were performed twice weekly for a 6-week-long period [17]. DEL-1-Fc treated sides, compared to Fc control-treated sites, displayed improved clinical indices of periodontal inflammation and tissue destruction over the course of the study and reduced bone loss at the end of the study. The inhibition of clinical inflammation correlated with significantly decreased abundance of proinflammatory and osteoclastogenic cytokines (IL-17, IL-1 β , TNF, and RANKL) in gingival crevicular fluid samples collected from DEL-1-treated sites, as well as with reduced neutrophil accumulation in the gingival tissue of DEL-1-treated sites. Moreover, the impaired bone loss correlated with reduced numbers of osteoclasts in biopsies of the bone from DEL-1-Fc-treated sites [17]. This effect could be attributed not only to the anti-inflammatory properties of DEL-1 (such as the inhibition of IL-17, which in turn induces RANKL that promotes osteoclastogenesis [76]) but also to the capacity of DEL-1 to directly downregulate the differentiation and activity of osteoclasts in a Mac-1 and α v β 3 integrin-dependent manner, respectively [17].

Multiple sclerosis and other IL-17-driven pathologies

As discussed earlier, DEL-1 deficiency enhances EAE severity and active human multiple sclerosis lesions are associated with reduced expression of DEL-1 [15]. Moreover, DEL-1-Fc circulates in the blood and exerts anti-inflammatory action in periodontitis (*i.e.*, its effectiveness does not necessarily require local administration) [14]. These observations provided the rationale to investigate whether systemic administration of DEL-1-Fc could provide a therapeutic benefit in multiple sclerosis, where therapeutic interventions are initiated after disease onset aiming to block or mitigate disease relapse. Therefore, the protective potential of DEL-1-Fc was tested in a relevant model, specifically the relapsing-remitting EAE model in SJL/J mice. Following the first clinical attack, the mice were given DEL-1-Fc or Fc control. Clinical relapse was prevented in DEL-1-Fc-treated mice as compared to controls [15]. Thus, systemic administration of DEL-1 represents a potential therapeutic approach in multiple sclerosis. The results from this preclinical model are consistent with the finding that *EDIL3* (gene coding for DEL-1) is a disease susceptibility gene in multiple sclerosis [23] and Alzheimer's disease [77]. Intriguingly, *EDIL3* has also been linked to ankylosing spondylitis [78], which is thought to be triggered by a dysregulated IL-23/IL-17 pathway [79] but mechanistic studies with regards to the role of DEL-1 in ankylosing spondylitis are missing. Together, the anti-inflammatory actions of DEL-1 are confined to IL-17-dependent inflammatory and autoimmune disorders, such as periodontitis, multiple sclerosis and perhaps ankylosing spondylitis or asthma (see above and ref. [16]), where IL-17 is pathologically implicated [80]. Hence IL-17-dependent pathologies represent the primary targets of DEL-1 based therapeutic applications.

HSC transplantation or hematological diseases

The discovery that DEL-1 is a crucial regulator of the HSC niche [20] has also major therapeutic implications, especially for HSC transplantation or hematological diseases. After transplantation, DEL-1 in the BM can promote the engraftment of hematopoietic progenitors and the reconstitution of myelopoiesis. Febrile neutropenia (which is associated with chemotherapy or HSC transplantation) is a life-threatening condition with high mortality [81]. DEL-1, therefore, could potentially be used for induction and maintenance of proper *de novo* myelopoiesis. In a different context, blockade of hematopoietic progenitor cell binding to DEL-1 may represent a therapeutic approach to promote progenitor cell mobilization, which would be beneficial for autologous or allogeneic HSC transplantation in hematologic malignancies.

Concluding remarks and future perspectives

The studies discussed in this review show that DEL-1 is flexibly down-regulated to facilitate immune responses when these are needed, but its expression resurges during inflammation resolution to critically promote efferocytosis and the plastic reprogramming of tissue macrophages to a pro-resolving phenotype [13,70]. Not only does DEL-1 act as a non-redundant downstream effector of inflammation resolution but is also required for optimal production of certain resolvins, possibly generating a positive-feedback loop that reinforces inflammation resolution [13] (Fig. 4).

Although DEL-1 was first established as a homeostatic regulator of IL-17- and leukocyte-mediated chronic inflammation in the setting of periodontitis, subsequent investigations showed that this concept applies also to diseases affecting tissues other than the periodontium, such as the central nervous system, the lungs and the airways, and the salivary glands [15,16,19,61]. Similarly, the identification of DEL-1 as a novel non-redundant factor of inflammation resolution working in synergy with resolvins [13] is expected to be relevant in additional disease settings (see Outstanding Questions).

The findings that endothelial cell-derived DEL-1 inhibits inflammation initiation, whereas macrophage-derived Del-1 promotes inflammation resolution [13] have revealed a new concept in the spatial regulation of the immune response, designated the ‘location principle’ (Fig. 4). The importance of the compartmentalized expression of secreted factors is also supported by another recent study showing that CXCL1 and CXCL2 from distinct cellular sources synergize by mediating non-redundant functions in the course of neutrophil transendothelial migration [73]. The differential functions of a secreted molecule may also depend on its intracellular versus extracellular localization, as was suggested for osteopontin [36]. In this context, a specific function of intracellularly localized DEL-1 has not been studied yet, and should be a topic of future research. It becomes evident that the expression of key multi-functional molecules at sites where they are specifically needed becomes a critical regulatory aspect of immune plasticity and homeostatic immunity. Future research therefore should be directed to identify additional such homeostatic molecules, understand their expression regulation and mechanisms of action, and exploit them from a therapeutic point of view (see Outstanding Questions).

Besides these local effects involving actions on mature myeloid cells (neutrophils, macrophages), DEL-1 has emerged as a novel and non-redundant regulatory factor of the BM niche that specifically instructs the generation of the myeloid progenitors through direct action on HSCs (**Fig. 1, left**), thereby contributing to innate immune cell replenishment in the context of infection/inflammation-induced demand myelopoiesis [20]. Hence, DEL-1 can modulate the host immune response both centrally and peripherally. Given the role of DEL-1 in promoting myelopoiesis, it would be worth analyzing its expression in the bone marrow in the context of hematologic malignancies and further hematologic conditions associated with cytopenias (see Outstanding Questions).

Because DEL-1 is an endogenous factor, the expression of which, at least in some cells/tissues, is reduced in inflammatory disorders, therapeutic interventions could aim at reconstituting its levels in order to restore its functions and thus promote tissue homeostasis (**Clinician's Corner**). Approval for therapies with endogenous molecule administration and/or replacement has increased in recent years, as it may bear less adverse effects. Examples of well-tolerated replacement of endogenous molecules include α_1 -antitrypsin given to α_1 -antitrypsin deficiency patients and digestive enzyme replacement in pancreas insufficiency [82]. Therefore, administration of DEL-1 to reinstate its diminished levels in inflammatory conditions may entail relatively minor safety issues, especially if it involves local administration (as is the case with periodontitis). In addition to periodontitis and EAE/multiple sclerosis that were discussed in detail here [14,15,17], DEL-1 was shown to protect also against pulmonary inflammation and fibrosis [19,25,59] and inflammatory reactions associated with islet transplantation [30] in preclinical models. DEL-1 is thus a therapeutically important regulatory molecule with multiple potential applications and merits further investigation in preclinical models in terms of basic biology and applied medicine.

In conclusion, Del-1 has emerged as an important homeostatic determinant of tissue immunity by acting in great part on neutrophils as DEL-1 (i) regulates their production in the bone marrow, (ii) controls their recruitment to inflamed tissues and (iii) promotes their clearance after they have performed their functions and have become apoptotic (Fig. 1).

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Glossary

Apoptosis

A form of programmed cell death, where the dying (apoptotic) cells are cleared without rupture and release of potentially harmful substances (*i.e.*, in a non-inflammatory manner).

Efferocytosis

(from *efferre*, Latin for 'to carry for burial') is the process by which dying (apoptotic) cells are removed through phagocytosis usually by macrophages or dendritic cells. Intracellular

signals, stimulated by the efferocytic process in the phagocyte, reprogram its transcriptional profile to assume a pro-resolving phenotype.

Emergency myelopoiesis

the generation of myeloid cells including neutrophils resulting from increased progenitor cell proliferation in the bone marrow in response to peripheral inflammatory stimuli or stress conditions, such as disseminated infection. This well-orchestrated process aims to meet the increased demand for myeloid cells during the innate immune response to the stress condition.

Gingival crevicular fluid

Serum exudate originating in the gingival capillaries and flowing into the gingival crevice (the space between the gingiva and the tooth surface); the fluid contains locally produced factors, such as immune and inflammatory mediators, together with large numbers of neutrophils recruited along a chemokine gradient.

Immune plasticity

the ability of immune cells to acquire divergent functional capabilities while maintaining a core transcriptional program. Immune plasticity contributes critically to tissue homeostasis and results from intimate interactions with tissue-derived factors.

Ligature-induced periodontitis model

an experimental periodontitis model involving the placement of a silk ligature around posterior teeth. Ligature placement simulates human periodontitis as it generates a subgingival biofilm-retentive milieu leading to inflammation and bone loss in conventional, but not germ-free, mice or other animals.

Location principle

the concept involving distinct and location-dependent homeostatic functions by the same multifunctional secreted molecule due to its compartmentalized expression, which can facilitate optimal performance with cell type-specific actions and spatiotemporal regulation of the immune response.

Hematopoietic stem cell (HSC) niche

the supportive microenvironment in the bone marrow where HSCs reside and self-renew. Endothelial and perivascular stromal cells as well as osteoblasts are important cell populations of the bone marrow niche and regulate HSC functions via adhesive interactions or paracrine growth factor and/or chemokine signals.

Homeostasis

a condition of equilibrium in a system that is sustained by adaptation of physiological processes to cope with external changes or challenges; for instance, a balance between endogenous factors in a host tissue and exogenous stimuli (pathogens, trauma, irritants) that prevents destructive inflammation or disease and thereby supports the maintenance of tissue integrity.

Multiple sclerosis

An immune-driven pathology of the central nervous system, which destroys myelin (the protective sheath that covers nerve fibers) and leads to a range of clinical symptoms, including sensory deficits and cognitive impairment.

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Box 1.**Periodontal disease**

Periodontitis is a prevalent chronic inflammatory disease characterized by destruction of the tooth-supporting bone and gingival tissue (collectively known as the periodontium). This oral disease is associated with enhanced risk for several chronic inflammatory disorders, including atherosclerosis and rheumatoid arthritis [83,84]. In its severe form, periodontitis afflicts nearly 10% of adults [85–87] and remains a formidable public health and economic burden [87]. Therefore, innovative host-modulation treatments are required to enhance existing therapies (*e.g.*, mechanical removal of the tooth-associated pathogenic biofilm), which are not always effective to control periodontitis [88].

Periodontitis is initiated by dysbiosis of the local microbiota, specifically by synergistic polymicrobial communities within which different species, or combinations of functional genes, synergize to collectively enhance colonization along with metabolic and immune subversion activities, leading to disruption of host-microbe homeostasis and dysregulated inflammation [89]. The destructive inflammatory response involves elements of both innate and adaptive immunity, such as complement, neutrophils, and IL-17 expressing CD4⁺ T cells (Th17), and culminates in pathologic activation of osteoclasts, which resorb the tooth-supporting bone [46,90,91]. Thus, if untreated, periodontitis can lead to tooth loss and possibly impaired mastication [92].

Periodontitis is characterized by defective resolution of inflammation [63,93], which thus perpetuates dysbiosis by providing nutrients (inflammatory tissue breakdown products) to the bacteria [43]. This generates a positive-feedback loop in which destructive inflammation and dysbiosis are reciprocally reinforced and the resulting ‘vicious cycle’ likely underlies the chronicity of this oral inflammatory disorder.

Box 2.**Inflammation resolution**

Successful resolution of inflammation involves a series of well-coordinated processes, including down-regulation of proinflammatory mediators, termination of further neutrophil recruitment, and clearance of apoptotic neutrophils through efferocytosis [94,95]. Critical for the process of efferocytosis is the recognition of the major ‘eat-me’ signal phosphatidylserine on the apoptotic cell surface by efferocytic receptors on macrophages or via the help of opsonins that bridge phosphatidylserine on apoptotic cells to macrophages [96].

The phagocytosis of apoptotic cells by macrophages not only prevents secondary necrosis and perpetuation of inflammation but also supports the plasticity of the efferocytic macrophage that adopts a resolving phenotype [94,95,97]. Specifically, upon efferocytosis, macrophages are reprogrammed to not only decrease production of proinflammatory cytokines but also to upregulate the expression of factors, such as TGF β and IL-10, with anti-inflammatory, immune modulatory and tissue remodeling actions, thereby restoring tissue integrity [13,94,96].

Successful inflammation resolution is also dependent on specialized pro-resolving mediators, which are derivatives of polyunsaturated fatty acids. Such mediators include the ‘resolvins’, which are synthesized from omega-3 fatty acids (D series resolvins from docosahexaenoic acid and E-series resolvins from eicosapentaenoic acid) and act through multiple mechanisms, such as, suppression of neutrophil infiltration, promotion of neutrophil apoptosis and stimulation of their efferocytosis by macrophages at the inflamed site [63,93,96]. Moreover, resolvins are crucial regulators of immune plasticity [98–100].

Box 3.**Clinician's Corner**

Immune-mediated diseases may not only be linked to the altered activation status of immune cells but may also arise from reduced levels or functional alterations of local tissue-derived homeostatic molecules leading to dysregulation of the initiation or termination of the host immune response.

Deficiencies in the homeostatic protein DEL-1 are causally linked to IL-17-dependent pathologies, such as inflammatory bone loss in periodontitis or experimental autoimmune encephalomyelitis/multiple sclerosis. Conversely, DEL-1 administration protects against these pathologies in preclinical non-human primate or murine models.

DEL-1 regulates the recruitment of neutrophils to infected or inflamed tissues. Although the expression levels of DEL-1 are diminished during the progression of inflammation, its expression is restored during the resolution phase. At this phase, DEL-1 promotes the efferocytosis of apoptotic neutrophils by macrophages, which are reprogrammed to acquire a pro-resolving phenotype.

The capacity of DEL-1 to regulate diverse and crucial activities that promote tissue homeostasis is attributed to its multi-functional structure but also to its compartmentalized expression that enables optimal performance with cell type-specific actions and spatiotemporal regulation of the immune response.

In addition to its local homeostatic effects in peripheral tissues, DEL-1 acts as a regulator of myelopoiesis in the bone marrow niche. Thus, DEL-1 can modulate the host immune plasticity both centrally and peripherally. This in turn indicates that the potential therapeutic applications of DEL-1 are not restricted to treatment of peripheral autoimmune and inflammatory diseases. DEL-1 could also be exploited in the context of bone marrow transplantation or hematological disorders.

Highlights

- DEL-1 has recently emerged as a critical factor for homeostatic immunity by regulating diverse functions, ranging from stress myelopoiesis in the bone marrow to initiation of local tissue inflammation and resolution thereof. In the bone marrow, DEL-1 acts as a niche factor that promotes myelopoiesis; endothelial-cell derived DEL-1 regulates neutrophil recruitment to the inflamed tissue; and macrophage-derived DEL-1 regulates efferocytosis and the plastic reprogramming of macrophages to a pro-resolving phenotype with enhanced expression of anti-inflammatory and tissue-remodeling factors.
- DEL-1 regulates neutrophils throughout their entire lifespan: It modulates the production and recruitment of neutrophils and the clearance of apoptotic neutrophils, depending on its expression location. DEL-1 deficiency is associated with several inflammation-driven pathologies.
- DEL-1 is a new and non-redundant member of a group of factors involved in resolution of inflammation. DEL-1 not only mediates efferocytosis and resolution of inflammation but also promotes production of resolvins, thereby generating a positive feedback loop that reinforces resolution.
- The spatial regulation of the immune response by DEL-1 reveals a new concept ('location principle') that is critical for immune plasticity and homeostatic immunity. Secreted molecules that exert location-dependent homeostatic functions may be important targets for therapeutic interventions in inflammatory and autoimmune diseases.
- DEL-1 is a paradigm of how local tissues regulate immune cells and influence health vs. disease as well as a potential therapeutic in inflammatory and autoimmune diseases.

Outstanding Questions

Can additional secreted molecules that exert location-dependent homeostatic functions be identified? What are their crucial structural features, mechanisms of action and tissue-specific / cell-specific expression pattern?

Do age-related alterations in expression of local tissue-derived homeostatic factors and/or their altered tissue compartmentalization / distribution contribute to the heightened chronic inflammatory state often associated with old age in humans ('inflamm-aging')?

Is the pro-resolution action of DEL-1, established in the periodontal and peritoneal compartments, relevant for inflammation resolution in other tissues?

Can DEL-1, or specific domains thereof, be exploited therapeutically for treating human inflammatory or autoimmune disorders?

Given the role of DEL-1 as a niche factor that promotes myelopoiesis, can this homeostatic protein find therapeutic application in hematologic conditions associated with cytopenias or hematologic malignancies?

Trends in Molecular Medicine

Figure 1. Del-1 regulates the production and recruitment of neutrophils and apoptotic neutrophil clearance.

In the bone marrow, DEL-1 acts as a niche factor that promotes myelopoiesis. Specifically, DEL-1 interacts with $\beta 3$ integrin on hematopoietic stem cells (HSCs) and induces HSC proliferation and biased differentiation toward the myeloid lineage (MyP, myeloid progenitors). In the vessel lumen, endothelial-cell derived DEL-1 blocks the interaction between LFA-1 ($\alpha L \beta 2$) integrin on neutrophils and ICAM-1 on the vascular endothelium, thereby inhibiting neutrophil adhesion and transmigration. DEL-1 can also block Mac-1 ($\alpha M \beta 2$) integrin, which mediates intraluminal crawling of neutrophils, although whether DEL-1 inhibits crawling has not been specifically addressed. Moreover, DEL-1 promotes macrophage efferocytosis and resolution of inflammation. Mechanistically, DEL-1 binds to the 'eat-me' signal phosphatidylserine (PS) on apoptotic cells as well as to the $\alpha v \beta 3$ integrin on macrophages, thereby acting as an efferocytic 'bridge' that enhances apoptotic cell phagocytosis. ECM, extracellular matrix; ICAM, intercellular adhesion molecule; PS, phosphatidylserine.

Figure 2. The DEL-1–IL-17 balance in immunity and inflammation.

In steady state, the reciprocal regulation between DEL-1 and IL-17 maintains a balance between the protective and detrimental effects of neutrophil recruitment thereby contributing to homeostatic immunity. However, in old age or in inflammatory disorders, the balance is tilted towards IL-17, which can directly downregulate DEL-1 expression, thereby potentiating neutrophil recruitment. Low levels or deficiency of DEL-1 is associated with enhanced IL-17 levels, thereby predisposing to IL-17-driven inflammatory disease (e.g., inflammatory bone loss).

Figure 3. IL-17 downregulates endothelial DEL-1 expression.

IL-17 (*e.g.*, derived from Th17 cells or $\gamma\delta$ T cells) interacts with the IL-17 receptor complex (IL-17RA/IL-17RC) and inhibits DEL-1 expression through a glycogen synthase kinase 3 β (GSK3 β)-dependent mechanism, which inhibits the binding of the critical transcription factor CCAAT/enhancer-binding protein β (C/EBP β) to the promoter of the DEL-1-encoding gene (*EDIL3*). This inhibitory action of IL-17 is counteracted at the GSK3 β level by the pro-resolving lipid mediator resolvin D1 (RvD1) in a phosphatidylinositol 3-kinase (PI3K)/Akt-dependent manner. This activity of RvD1 (which may derive from resolving macrophages) is mediated through interactions with specific receptors, the G-protein-coupled receptor 32 (GPR32) and the lipoxin A4 receptor/formyl-peptide receptor-2 (ALX/FPR2).

Figure 4. Location principle.

Endothelial cell-derived DEL-1 primarily regulates inflammation initiation by restraining LFA-1/ICAM-1-dependent neutrophil recruitment, whereas macrophage-derived DEL-1 promotes the resolution of inflammation. In this regard, DEL-1 enhances efferocytosis and LXR-dependent reprogramming of the efferocytic macrophage that can upregulate pro-resolving factors, such as TGF β and resolvins. Resolvins may contribute to the rising levels of DEL-1 during resolution, for instance, by counteracting inhibitory mechanisms that would otherwise suppress DEL-1 expression. Together, these observations suggest the operation of a feed-forward loop that links DEL-1 and resolvins and reinforces inflammation resolution. These location-dependent homeostatic functions facilitate the spatiotemporal regulation of the immune response by the same multifunctional molecule, DEL-1. ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; LXR, liver X receptor; PS, phosphatidylserine.