

## Theme Issue Article

# Neutrophils launch monocyte extravasation by release of granule proteins

Oliver Soehnlein; Alma Zerneck; Christian Weber

Institute for Molecular Cardiovascular Research (IMCAR), RWTH University Aachen, Germany

## Summary

During their journey from the blood stream to sites of inflammation polymorphonuclear leukocytes (PMN) release a wide panoply of granule proteins. Shortly after the PMN efflux, the extravasation of monocytes sets in and recent research provides evidence that the release of PMN granule proteins and monocyte extravasation are causally interrelated. Granule proteins seeded on the endothelium by adherent PMN allow direct activation and subsequent adhesion of monocytes. In addition, PMN granule components enhance the endothelial expression of cell adhesion molecules, efficiently supporting the arrest of mono-

cytes at inflamed vessels. Moreover, granule proteins contribute to the fine tuning of the local chemokine network. Proteolytic modification of chemokines as well as enhancement of local chemokine synthesis lead to increased monocyte extravasation. Finally, PMN granule proteins exert direct chemotactic effects, a mechanism which is of special importance in the early recruitment of inflammatory monocytes. Hence, granule proteins modify the monocyte extravasation cascade in a multifaceted manner ensuring the efficiency of these mechanisms.

## Keywords

Inflammation, neutrophil, monocyte, extravasation, chemokine

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## Extravasation of PMN precedes a second wave of monocytes

In many inflammatory conditions polymorphonuclear leukocytes (PMN) dominate the initial leukocyte influx into the inflamed tissue. This first wave of PMN extravasation is soon replaced by a second wave of emigrating monocytes. Initial proof for this sequence of events was obtained from observations by Rebeck and Crowley (1). In this early study, PMN dominate leukocyte extravasation at three hours after initiation of the inflammation. However, at later time points the cellular composition of extravasated cells is predominated by monocytes (1). In subsequent years Ward et al. demonstrated that PMN lysate efficiently attracts monocytes (2), indicating (i) that there may be a causal link between the sequence of events in leukocyte extravasation and (ii) that a preformed component in the PMN may induce monocyte extravasation. It was not until 1982 that substantial proof came as to what PMN component(s) may be attributable for the chemotactic effect on monocytes. Gallin et al. found

that the PMN lysate of patients suffering from specific granule deficiency (SGD) lacks its chemotactic effect on monocytes (3). PMN from SGD patients are deficient of granule proteins such as human neutrophil peptides (HNPs,  $\alpha$ -defensins) and hCAP-18 (pro-form of LL-37) (4), indicating an importance of these granule components in attracting monocytes. Confirmation for the relevance of individual PMN granule proteins came from studies by Oppenheim and colleagues identifying a direct chemotactic potency of certain PMN granule proteins (5–7). In this review we focus on mechanisms by which PMN granule proteins contribute to the extravasation of monocytes.

## PMN granule subsets

Granule proteins take center stage in the communication of the PMN with its surrounding. Preformed and stored in vesicles, granule proteins are at the PMN's immediate demand during the inflammatory response. Different granule subsets with specialised functions can be distinguished (8). Secretory vesicles have

### Correspondence to:

Oliver Soehnlein, MD, PhD  
IMCAR, RWTH University Aachen  
Pauwelsstr. 30  
52074 Aachen, Germany  
Tel.: +49 241 80 36496, Fax: +49 241 80 82716  
E-mail: osoehnlein@ukaachen.de

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Table 1: PMN granule composition.

	Primary granules	Secondary granules	Tertiary granules	Secretory vesicles
Membrane	CD63, CD68	Laminin-R, Vitronectin-R, TNF-R, FPR	CD11b, FPR	CD11, CD14, CD16, FPR, CD10, CD13
Matrix	azurocidin, cathepsin G, elastase, proteinase-3, HNPI-3, MPO	lactoferrin, hCAP18 (LL-37), NGAL	MMP-9, lysozyme	azurocidin, proteinase-3, albumin

the highest propensity for release and conclusively their contents are delivered to the endothelium during the initial interaction of the PMN with the vessel wall. **Tertiary granules** are released during PMN transmigration across the endothelial lining. Contents in this compartment are rich in proteases such as MMP-9 (Table 1) allowing the PMN to cut through the basement membrane more efficiently. **Primary** and **secondary granule** contents are released in the extravascular space. Antimicrobial polypeptides in these compartments (Table 1) are important in bacterial clearance. They do so by direct antimicrobial activity (9), bacterial opsonisation (10, 11) and macrophage activation (12, 13). In addition, many of these antimicrobial proteins also contribute directly to the activation of subsequent immune mechanisms and were therefore termed 'alarmins' (14–16).

## Mechanisms of monocyte extravasation

The extravasation of monocytes from the vascular lumen to the tissue involves a series of sequential molecular interactions between monocytes and endothelial cells. Selectins allow the monocyte to bind weakly and initiate the adhesion cascade. The rolling monocyte is then stimulated – by chemokines or other chemotactic compounds, such as PAF or LTB<sub>4</sub> – to engage its surface integrins e. g.  $\beta_1$ - and  $\beta_2$ -integrins with counter-receptors expressed by endothelial cells. This results in firm adhesion of monocytes to the endothelium before they emigrate through the vessel wall. Chemokines subsequently lead to the induction of active, cytoskeleton-driven transendothelial migration and extravasation (17–19). Chemokines are small chemotactic cytokines that can be secreted by endothelial cells, platelets, leukocytes or stromal cells (19–21). Several chemokines can bind transmembrane heparin sulfate proteoglycans on the luminal surface of vascular endothelial cells and thus are presented to leukocytes (22–25). When chemokines bind to these proteoglycans, the binding site for the specific G protein-coupled chemokine receptor remains exposed. Thus, when a rolling monocyte encounters a chemokine bound to the endothelium, the chemokine interacting with its receptor on the monocyte surface elicits a rapid integrin-activation signal.

The monocyte population is a heterogeneous set of peripheral white blood cells (26–28). In the human system these subsets have distinct functional differences with classical CD14<sup>+</sup>CD16<sup>-</sup> monocytes producing higher amounts of cytokines and contribute more effectively to bacterial clearance by phagocytosis as compared to non-classical monocytes (29–31). In contrast, non-classical CD14<sup>lo</sup>CD16<sup>+</sup> monocytes are more potent in presenting antigens (26). While migration of CD14<sup>+</sup>CD16<sup>-</sup> monocytes into

sites of inflammation has been shown to be governed by CCL2 (32), CD14<sup>lo</sup>CD16<sup>+</sup> failed to migrate in response CCL2, consistent with the absence of CCR2 on these cells (33). Studies by Ancuta et al. (34) showed that the CD14<sup>lo</sup>CD16<sup>+</sup> monocytes will migrate in response to CX3CL1 and CXCL12. It was also noted, however, that the CD14<sup>lo</sup>CD16<sup>+</sup> monocytes adhere to activated endothelium more strongly (35), and this was suggested to be mediated in part by CX3CL1 expressed on the cell surface of the endothelial cells. Such firm adherence to CX3CL1-expressing endothelial cells did lead to a reduced transmigration in response to CX3CL1 *in vitro*.

While it has been known for many years that human peripheral blood monocytes are a heterogeneous population of leukocytes, distinguishable by the expression of CD14 and CD16, this has just recently also been confirmed in the murine circulation. Geissmann et al. described the existence of at least two different monocyte subsets, which can be distinguished by their expression levels of Ly-6C (or Gr1) and of the chemokine receptors CCR2, and CX<sub>3</sub>CR1 (36). Murine CX<sub>3</sub>CR1<sup>lo</sup>CCR2<sup>+</sup>Gr1<sup>+</sup> monocytes share morphological characteristics and chemokine receptor expression patterns with the classical human CD14<sup>hi</sup>CD16<sup>-</sup>, while CX<sub>3</sub>CR1<sup>hi</sup>CCR2<sup>-</sup>Gr1<sup>-</sup> are thought to resemble the phenotype of non-classical human CD14<sup>lo</sup>CD16<sup>+</sup> monocytes. These subsets were furthermore proposed to employ different mechanisms for extravasation and possibly exert also different functions with Gr1<sup>+</sup> monocytes termed 'inflammatory' and Gr1<sup>-</sup> as 'resident' monocytes accordingly. Because Gr1<sup>+</sup> monocytes express CCR2, a molecule well known to be involved in inflammatory monocyte recruitment (37), it was proposed that Gr1<sup>+</sup> monocytes are rapidly recruited to sites of inflammation. Indeed, in typical models of acute inflammation, Gr1<sup>+</sup> monocyte recruitment is critically dependent on CCR2 (38, 39), but not on CX<sub>3</sub>CR1 (40). The latter has just recently been shown to be important in monocyte homeostasis by promoting cell survival (41). In addition to CCR2, CCR6/CCL20 is involved in the recruitment of Gr1<sup>+</sup> monocytes (42, 43).

Less is known about the trafficking and fate of Gr1<sup>-</sup> monocytes. In contrast to Gr1<sup>+</sup> monocytes, they migrate scarcely or not at all to inflamed tissue in mice, including the acutely inflamed peritoneum (36, 44), or to skin after intracutaneous injection of latex beads, administration of vaccine formulations (43), or epicutaneous ultraviolet light exposure (45). It has therefore been hypothesised that Gr1<sup>-</sup> monocytes may fulfill critical roles in replacing resident macrophages or dendritic cells (DC) in the steady state (36). It has further been suggested that the Gr1<sup>-</sup> monocytes utilise CX<sub>3</sub>CR1 to migrate into non-inflamed tissue for replacing resident macrophages or DC, given their higher

surface expression of CX<sub>3</sub>CR1 and the CX<sub>3</sub>CL1-dependent transendothelial migration of the corresponding human subset of CD16<sup>+</sup> monocytes *in vitro* (34, 36). However, the experimental evidence for the trafficking pattern and the potential role of Gr1<sup>+</sup> monocytes as precursors for resident tissue cells is rather limited. Just recently Auffray et al. demonstrated that Gr1<sup>+</sup> monocytes patrol healthy tissue by long-range crawling on resting endothelium. This crawling depends on LFA-1 as well as CX<sub>3</sub>CR1 (46). Functionally, the patrolling was thought to allow for rapid extravasation upon injury. However, further studies are needed to corroborate these findings and to elucidate the functional properties of this monocyte subset.

## How do PMN granule proteins contribute to the extravasation of monocytes (Fig. 1)?

### I. PMN-derived granule proteins directly induce monocyte adhesion – focus on azurocidin and proteinase-3

The monocyte extravasation cascade involves a series of sequential non-redundant ligand-receptor interactions between monocytes, endothelial cells, and chemoattracting agents engaging selectins, cell-adhesion molecules and chemokines. Lack in just one of these components will abrogate the process of monocyte recruitment. Intravital microscopic observations indicate that depletion of PMN reduces the number of adherent monocytes in postcapillary venules of the inflamed cremaster muscle (47). In this early process of monocyte extravasation PMN granule proteins may enhance monocyte adhesion in two ways – either granule proteins anchor on endothelial cells and in this position allow for monocyte activation with a subsequent binding to CAMs or possibly also the granule protein itself. Alternatively, granule proteins directly augment the expression of endothelial CAMs (see paragraph II). Components of secretory vesicles interact closely with the endothelium and therefore play an important role in monocyte adhesion. Unlike other granule compartments, secretory vesicles are rich in membrane bound receptors, while soluble proteins are scarce (Table 1). Besides serum proteins which point at the vesicles' endocytotic origin, only azurocidin and proteinase-3 were shown to reside within this compartment (48, 49). Azurocidin is liberated from PMN after  $\beta_2$ -integrin ligation and due to its charge anchors on endothelial cells. Interestingly, azurocidin deposition was not observed when firm adhesion of PMN was blocked, indicating that a transient PMN-endothelium interaction is not sufficient for release of secretory vesicles. For its cationicity azurocidin interacts with negatively charged proteoglycan side chains on endothelial cells (50, 51) and is thereby presented to immune cells in the blood flow. Due to the localisation in the same compartment and their homology, similar mechanisms may also be true for proteinase-3 (52, 53). However, most of the proteinase-3 remains associated with the PMN surface after mobilisation of secretory vesicles. Therefore lower amounts of proteinase-3 may be deposited on the endothelial cell surface. Immunohistochemical staining for azurocidin and proteinase-3 revealed their localisation in human specimens in both acutely or chronically inflamed tissues. For example, azurocidin and proteinase-3 were demonstrated on the endothelial cell surface of atherosclerotic plaques as well as in lesions

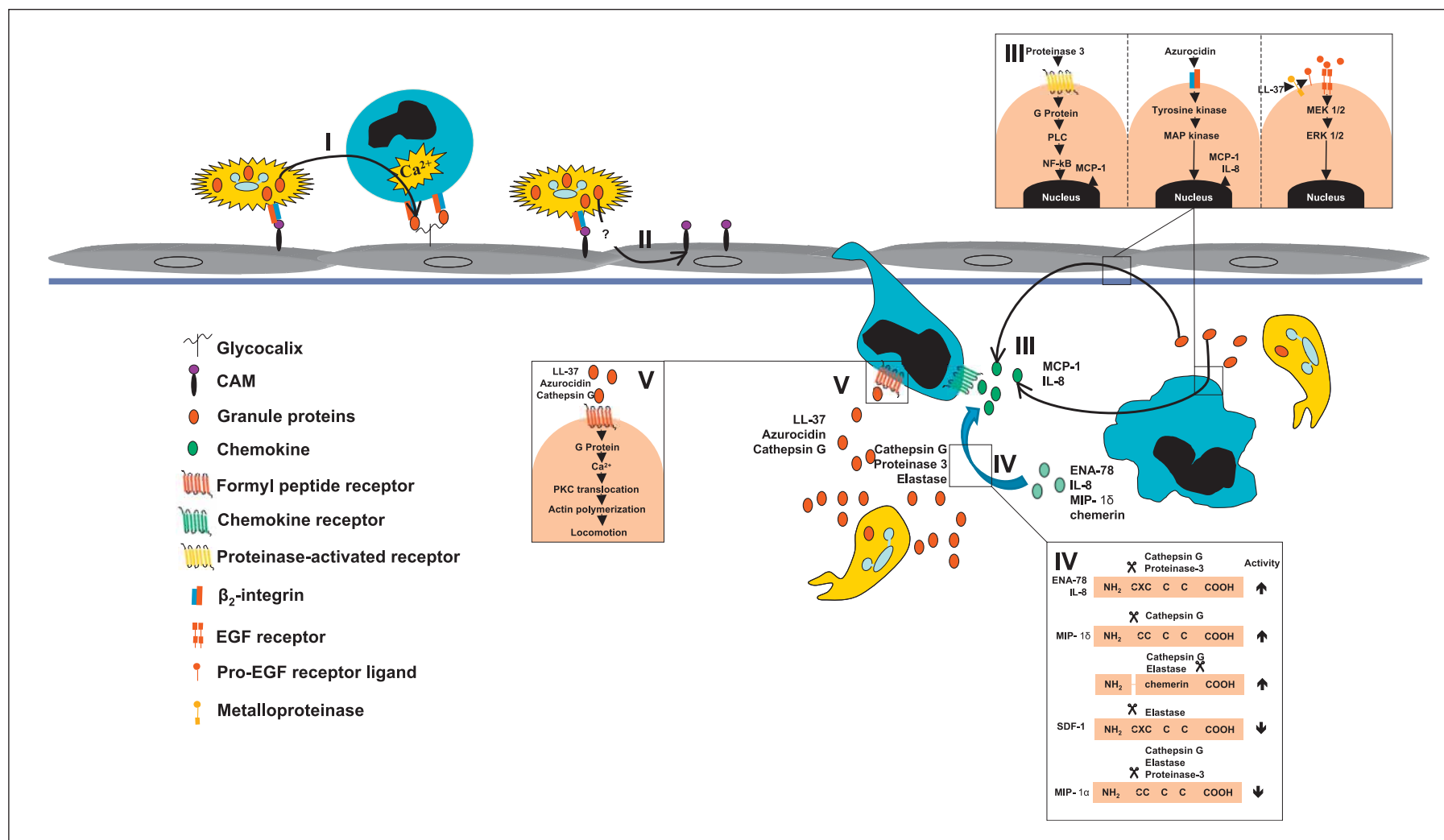
from Alzheimer's patients (54, 55). In this location azurocidin is prone to activate monocytes and specifically induces their adhesion. This is in line with observations that fluorescein isothiocyanate (FITC)-conjugated azurocidin binds to monocytes, but not lymphocytes and to a lower degree to PMN (56, 57). Unpublished data indicate that the adhesion of monocytes to azurocidin is mediated via  $\beta_2$ -integrin activation. Similarly, leukocyte adhesion to proteinase-3 has been shown to be mediated via binding to  $\beta_2$ -integrins (58). In addition, it was also demonstrated that neutrophil elastase binds to the endothelial cell surface and in this location induces leukocyte adhesion via involvement of  $\beta_2$ -integrins. However, since neutrophil elastase is exclusively located in primary granules, which are discharged once the PMN has reached the extravascular tissue, the importance of neutrophil elastase in mediating monocyte adhesion remains unclear. A comparable mechanism has recently also been shown for the arrest chemokine RANTES (regulated on activation normal T cell expressed and secreted). Von Hundelshausen et al. demonstrated that RANTES from platelet granules may be deposited on endothelial cells enhancing the adhesion of monocytes (59). Both PMN granule proteins and the chemokine RANTES seeded on the endothelium are not derived from the inflammatory lesion itself but from a blood cell, proposing a novel concept in monocyte biology.

### II. PMN-derived granule proteins enhance CAM expression – focus on azurocidin and proteinase-3

The interaction of granule proteins with the endothelium is, however, not as passive as it may seem. In fact, PMN granule proteins are potent activators of endothelial cells. Azurocidin for instance induces rapid Ca<sup>2+</sup>-mobilisation in endothelial cells resulting in vascular leakage (60–62). Azurocidin also activates endothelial PKC (63) and is rapidly internalised after the initial attachment to the endothelial cell. The latter mechanism may be important in preventing endothelial cell apoptosis (50) while the first may be important for the altered gene expression observed after exposition of endothelial cells to azurocidin (64). Lee et al. have shown that azurocidin enhances the expression of VCAM-1 and ICAM-1, resulting in enhanced adhesion of monocytes (64). Similarly, proteinase-3 induces expression of these two CAMs resulting in enhanced adhesion of PMN and monocytes to isolated endothelial cells (65). In addition, recent work showed that also HNP1–3 or LL-37 from PMN induce a severalfold increase in VCAM-1 expression on endothelial cells (66, 67). However, since these two polypeptides are normally released at later stages of PMN extravasation it remains unclear in what way these small peptides contribute to monocyte adhesion in a more physiological setting.

### III. PMN granule proteins induce chemokine expression – focus on LL-37 and HNP3s

Activation of endothelial cells may not only lead to enhanced CAM expression but also to increase in the release of chemokines. In this context, PMN-derived proteinase-3 was shown to induce the secretion of MCP-1 (CCL2) from endothelial cells (65), which via its receptor CCR2 expressed on inflammatory monocytes, induces their recruitment. In contrast to these findings, PMN-derived azurocidin has not been shown to induce che-



**Figure 1: Synopsis of mechanisms by which PMN granule proteins contribute to the recruitment of monocytes.** (I) Adherent PMN release granule proteins that bind to the endothelial glycocalix. In this location, granule proteins directly activate monocytes which may lead to monocyte arrest. Adhesion in response to deposited azurocidin and proteinase-3 involves activation of  $\beta_2$ -integrin. (II) In addition, granule proteins may enhance CAM expression contributing to the firm adhesion of monocytes. The exact mechanism by which granule pro-

teins enhance CAM expression is unknown. (III) Granule proteins liberated from extravasated PMN activate neighboring cells to express chemokines. (IV) In such location granule proteins also cleave chemokines, thereby enhancing or decreasing their ability to chemoattract monocytes. (V) Finally, PMN granule proteins exert direct chemotactic activity for monocytes, much of which is mediated via FPRs.

**Table 2: Concentration of PMN granule proteins in the supernatant of activated PMN.** Indicated are the concentrations of PMN granule proteins in the supernatant of PMN that were activated by antibody cross-linking of CD18. The concentration of granule proteins *in vivo* was assessed in the peritoneal lavage fluid from mice receiving thioglycollate intraperitoneally.

Protein	Concentration <i>in vitro</i>	Concentration <i>in vivo</i>
Azurocidin	3.4 µg/ml	
Cathepsin G	2.1 µg/ml	
HNPs	2.5 µg/ml	
LL-37	3.1 µg/ml	
MMP-9	1.7 U/ml	0.43 U/ml
MPO	1.3 U/ml	0.55 U/ml

monokine release from endothelial cells but from monocytes. This mechanism may be favored by azurocidin's localisation in not just secretory vesicles, but also in primary granules (16, 48). Azurocidin released from the latter compartment enhances the release of MIP-1 $\alpha$  and IL-8 from LPS-primed monocytes (68, 69). A more complex mechanism for the induction of IL-8 synthesis has been shown for LL-37 in epithelial cells. Here, epidermal growth factor receptor (EGFR), which is present on airway epithelial cells, is transactivated by LL-37 via metalloproteinase-mediated cleavage of membrane anchored EGFR ligands. Downstream signalling involves the MAPK/ERK pathway, leading to release of the potent chemoattractant IL-8 (70). Similar results were obtained for monocytes, showing that LL-37 in these cells mediates the induction of IL-8 via a G-protein coupled independent receptor, activating ERK and p38 pathways (71).

Consistent with results for LL-37, HNP-1 induces IL-8 and MCP-1 production in epithelial cells (72–74). In addition, epithelial cells produce IL-1 $\beta$  in response to HNP-1. The effect of HNP-1 appears to be selective, as the transcription of other genes, e. g. TNF $\alpha$ , GM-CSF, MCP-1, TGF- $\beta$ 1 and VEGF (vascular endothelial growth factor), and the release GM-CSF, IL-1 $\beta$  and TGF- $\beta$ 1 remain at levels comparable to untreated control cells. While there has been substantial research demonstrating chemokine and cytokine production in response to defensins, and HNP-1 in particular, the mechanism of induction is not well understood. In part, these effects might be mediated by activation of the transcription factor NF- $\kappa$ B. In addition, it was recently demonstrated that IL-8 production in response to HNPs might be mediated through the purinergic P2 receptor P2Y<sub>6</sub> receptor (75). In line, while treatment with ATP or UDP, known ligands for P2Y<sub>6</sub>, selectively induced the release of IL-8, the HNP-1 induced production of IL-8 could be abrogated in the presence of P2Y<sub>6</sub>-antisense but not P2Y<sub>6</sub>-sense oligonucleotides (75).

#### IV. PMN granule proteins activate chemokines – focus on cathepsin G, elastase, and proteinase-3

While the family of PMN-derived serprocidins (serine proteases with antimicrobial activity) has not been shown to be involved in chemokine synthesis, their members may contribute to the chemokine network by proteolytic mechanisms. Indeed, many cyto-

kines and chemokines and their respective receptors contain putative cleavage sites for neutrophil serine proteases. It is therefore not surprising that many receptors, cytokines and other molecules have been found to be natural substrates for neutrophil serine proteases (76, 77). It has been shown that the N-terminal processing of certain chemokines increases their affinity for their receptor. Thus, N-terminal cleavage of IL-8 by proteinase-3 (78) and ENA-78 by cathepsin G (79) releases truncated forms of these chemokines that have higher chemotactic activity than the full-length molecules. Similarly, N-terminal modification of MIP1 $\delta$  (CCL15) by cathepsin G increased its monocyte chemotactic activity manifold (80). Recently, it has been shown that activation of chemerin, which is known to attract APCs, can be mediated by neutrophil elastase and cathepsin G through the proteolytic removal of a C-terminal peptide (81). However, N-terminal truncation of a chemokine through proteolysis does not always lead to increased cellular activation. Processing of SDF-1 $\alpha$  by neutrophil elastase (82) and proteolysis of MIP1 $\alpha$  isoforms by all three neutrophil serine proteases (83) was shown to result in loss of chemotactic activity. Therefore, the net effect of the proteolytic modification of chemokines by neutrophil serine proteases *in vivo* remains unclear.

Neutrophil serine proteases have also been implicated in the activation of the pro-forms of TNF $\alpha$  and IL-1 $\beta$  (84–87). TNF $\alpha$  is proteolytically released from membrane-bound pro-TNF by the metalloproteinase TNF-converting enzyme. Similarly, IL-1 $\beta$  is converted to its active form by IL-1 $\beta$ -converting enzyme. However, it has been shown that proteinase 3 can also process pro-TNF to a biologically active soluble form and directly activate IL-1 $\beta$ .

#### V. PMN granule proteins directly chemoattract monocytes – focus on LL-37, azurocidin, cathepsin G, and HNPs

Once the monocyte has adhered it may start to transmigrate given the presence of appropriate signals. Several PMN-granule proteins were shown to exert chemotactic effects on monocytes *in vitro*. In this respect, the secondary granule-derived LL-37 and the primary granule-derived azurocidin, cathepsin G, and HNP1–3 were demonstrated to be chemotactic for monocytes (5–7, 14–16). PTx-sensitivity of these events points at the involvement of G-protein coupled receptors. Further research elucidated that cathepsin G activates human FPR, while LL-37 acts via FPRL-1 (7). Also azurocidin was recently shown to induce monocyte extravasation via FPRs (47). However, the mechanisms and receptors underlying the monocyte extravasation triggered by HNPs still remain unknown. Due to their similarity, HNPs are often grouped according to their functions. However, the cell types attracted by that individual HNPs are still under debate. For example, HNP-1 chemoattracts monocytes, naïve T cells and immature DC, while it conversely inhibits the attraction of PMNs, as well as their migration in response to formyl-methionyl-leucyl-phenylalanine (fMLP) and leukotriene B<sub>4</sub> (5, 6, 14, 15, 88). This inhibition appears to be specific as HNP-1 was not able to inhibit the recruitment of PMNs in response to IL-8. Additionally, whereas HNP-2 chemoattracts naïve T cells and immature DC as well as chemoattracts monocytes, HNP-3 does not have any of these activities. That monocyte chemoattraction by

PMN granule proteins is also relevant *in vivo* is further supported by the concentration these proteins reach in the tissue (Table 2). While it is difficult to assess the concentration of HNP in murine tissue due to species differences, the analysis of marker proteins like MPO and MMP-9 revealed that these were in the same range in the inflamed peritoneum as in the supernatant of activated PMN (12).

It is important to note that phenotypic differences of various monocyte subsets encompass distinct recruitment mechanisms, very likely reflected also in a specific recruitment of certain monocyte subsets by PMN. In this context it has recently been demonstrated that depletion of PMN specifically reduces the recruitment of Gr1<sup>+</sup> monocytes (47). Interestingly, this deficiency in recruitment could almost completely be rescued by the local application of the supernatant from activated human PMN. In subsequent experiments LL-37 and azurocidin were identified as principal mediators of this effect, both of which activate Gr1<sup>+</sup> monocytes via FPRs. The functional importance of Gr1<sup>+</sup> monocytes has just recently been shown for the rehabilitation after muscle injury (89) and myocardial infarction (90), for the control of acute toxoplasma infection (91), but also for the development of atherosclerosis (92). PMN granule proteins may act as a major source for chemotactic substances which ignite the recruitment of inflammatory monocytes. Therefore, PMN may also be of importance in pathological conditions as those mentioned above. At later time points, additional mechanisms, such as chemokine release from PMN (93) or other immune cells (94), likely gain increasing importance for attracting monocytes to the inflamed tissue.

### How important are such mechanisms *in vivo*?

The physiological importance of PMN and their granule proteins for subsequent monocyte recruitment becomes evident in clinical conditions such as neutropenia, PMN granule deficiencies, as well as PMN dysfunctions. In animal models, a reduction in monocyte extravasation is observed when inducing neutropenia by injection of a PMN-depleting antibody, a mechanism which may in part be responsible for the frequent pyogenic infections in neutropenic patients (95, 96). In addition, SGD patients exhibit obvious changes in monocyte and macrophage function. As such, macrophage maturation, migratory capacity, cytokine gene expression, and phagocytosis are affected in both humans and mice. In the case of dipeptidyl peptidase I (DPPI) deficiency resulting in a loss of function of the PMN serine proteases elastase, cathepsin G and proteinase-3 a secondary macrophage dysfunction, and a reduced monocyte accumulation are observed (97).

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Most of these mechanisms seem to be favored in acute bacterial infections. However, it has just recently been demonstrated that the PMN may also be of importance in the initiation and progression of atherosclerosis (98). PMN are not just located within the atherosclerotic plaque, but also deposit their granule proteins within atherosclerotic lesions. Recent advances suggest a functional significance of the PMN and its secretion products in the development of this chronic inflammation (99). Atherosclerosis prone apolipoprotein E-deficient mice, which were rendered neutropenic for four weeks, developed significantly less atherosclerosis than mice with normal white blood cell counts, which was furthermore associated with a lower number of macrophages in the atherosclerotic lesion. Thus, mechanisms as described above may not just be important in acute bacterial infections but also in chronic sterile inflammatory responses.

### Concluding remarks

PMN and their granule proteins are important regulators of the extravasation of monocytes. Since granule proteins are readily available during the PMN extravasation process, these are of significance in the early recruitment of monocytes. In this, PMN granule proteins mediate monocyte adhesion by deposition of granule proteins and enhancement of endothelial CAM expression. The subsequent monocyte extravasation is driven by activation and production of chemokines in response to granule proteins. Finally, granule proteins directly exhibit a chemotactic effect on monocytes, much of which is mediated via FPRs. While it is unknown if granule proteins preferably mediate adhesion of a distinct monocyte subset, it has emerged that certain granule proteins specifically contribute to the extravasation of Gr1<sup>+</sup> inflammatory monocytes. The multifaceted action of PMN granule proteins in boosting the inflammatory response and specifically the recruitment of monocytes may therefore offer a powerful target to interfere with the extravasation of monocytes.

### Abbreviations

CAM, cell adhesion molecule; DPPI, dipeptidyl peptidase I; EGFR, epidermal growth factor receptor; fMLP, formyl-methionyl-leucyl-phenylalanine; FPR, formyl peptide receptor; HNP, human neutrophil peptide; IL, interleukin; LTB<sub>4</sub>, leukotrien B<sub>4</sub>; MCP, monocyte-chemotactic protein; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NFκB, nuclear factor kappa B; PAF, platelet-activating factor; PMN, polymorphonuclear leukocyte; SDF, stromal cell-derived factor; SGD, specific granule deficiency; TGF, transforming growth factor; TNF, tumor necrosis factor.

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