25

IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation

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The transition from neutrophil to mononuclear-cell infiltrate is a hallmark of acute inflammation. The kinetics of leuko-endothelial adhesion molecule expression and chemokine secretion might participate in the initial recruitment of neutrophils. Neutrophils then die in situ by apoptosis, while mononuclear cells accumulate. We propose that interleukin-6 (IL-6) and its soluble receptor (slL-6R α) might regulate the leukocyte recruitment transition, through a shift of chemokine production. This new function of IL-6 might aid in the understanding of its complex role in inflammation: during acute inflammation, IL-6 might favor the resolution of the neutrophilic infiltrate and the initiation of the immune response; in chronic inflammation, IL-6 might increase the mononuclear-cell infiltrate and participate in disease pathogenesis.

Inflammation is a complex defense mechanism characterized by leukocyte migration from the vasculature into damaged tissues to destroy the injurious agents. Acute inflammation is a limited beneficial response particularly during infectious challenges, whereas chronic inflammation is a persistent phenomenon, which can progress to inflammatory diseases. One hallmark of acute inflammation is that the leukocyte infiltrate is initially mostly neutrophilic but after 24 to 48 h, monocytic cells predominate [1-3]. By contrast, chronic inflammation is histologically associated with the presence of mononuclear cells, such as macrophages and lymphocytes [1,2]. Although several explanations have been suggested, the mechanisms controlling the transition from neutrophil to monocyte recruitment during acute inflammation are poorly known. Here, we review different possible mechanisms involved and propose that the IL-6–soluble IL-6R α $(sIL-6R\alpha)$ complex might have an important role in this transition.

Why are polymorphonuclear (PMN) cells the first leukocytes recruited to the inflammatory site? In several different models of acute inflammation, neutrophils are the first cells to accumulate in tissues [1-3]. One explanation for the initial recruitment of PMN cells might be the high concentration of these cells in the blood compared to monocytes. Another explanation could be the kinetics of expression of leuko-endothelial adhesion molecules. Adhesion of leukocytes to endothelium is a multistep process characterized by an initial weak interaction mediated by selectins and their carbohydrated ligands, giving a rolling motion to leukocytes. Rolling enables leukocyte activation by chemotactic agents associated with the endothelial-cell membrane, inducing activation of β integrins on the white-cell surface. These molecules interact with endothelial members of the immunoglobulin superfamily for firm leuko-endothelial adhesion [4,5]. Leukocytes then begin to cross the endothelial layer through homologous interactions of PECAM-1 [platelet endothelial-cell adhesion molecule (CD31)] expressed on both leukocytes and the intercellular membranes of endothelial cells, and migrate following a chemoattractant gradient initiated in the injured tissue [6]. Neutrophil adhesion is mainly supported by the selectins CD62L on white cells and CD62P on endothelium, the leukocyte $\beta 2$ integrins CD11a/b (CD18) and the two endothelial members of the immunoglobulin superfamily, intercellular adhesion molecule-1 (ICAM-1) and ICAM-2. Each of these molecules are either constitutively present on leukocytes and endothelium, such as CD62L, CD11a (CD18), ICAM-1 and ICAM-2 or are very rapidly translocated, activated and upregulated, for example, CD62P, CD11b (CD18) and ICAM-1. Moreover, neutrophil recruitment is facilitated by chemotactic agents, such as C5a, leucotriene B4 (LTB4) or the platelet activating factor which are synthesized in minutes. In addition, the chemokine IL-8 (CXCL8), which is important for neutrophil recruitment in vivo, exists as a preformed pool in endothelial cells [7]. By contrast, the endothelial selectin CD62E, the β 1 integrin VLA-4 on leukocytes and the endothelial immunoglobulin superfamily member vascular-cell adhesion molecule-1 (VCAM-1), which are more important for monocyte adhesion, are absent from the surface of quiescent leukocytes or endothelium and require several

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Opinion

Type of cells	Production of IL-6 ^a	Expression of IL-6Rα	Expression of gp130	Effects of IL-6
Neutrophil	Yes	Yes	Yes	Increases elastase, PAF, IL-1Ra secretion, phagocytosis (at high concentrations) and apoptosis
Monocyte Macrophage	Yes	Yes	Yes	Favors differentiation into macrophages, increases oxydative burst, tissue factor expression, MCP-1, IL-1Ra production, decreases IL-1 β , TNF- α , and IL-12 production
Endothelial cell	Yes	No	Yes	Increases ICAM-1 expression (+/- VCAM-1, CD62E), MCP-1, IL-6 (+/- IL-8) production and protein S synthesis
Fibroblast	Yes	No or weak	Yes	Increases or decreases proliferation, increases collagen, glycosaminoglycan and TIMP-1 synthesis, MCP-1 and IL-6 production
Dendritic cell	Yes (mature cells)	No	Yes	Increases precursor – cell proliferation, phagocytosis, naïve T-cell attraction, GM-CSF receptor expression, IL-12 secretion

Table 1. Effects of IL-6 on various inflammatory cells^a

^aAbbreviations: GM-CSF, granulocyte-macrophage-colony stimulating factor; ICAM, intercellular adheson molecule; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; MCP, monocyte chemotactic protein; PAF, platelet activating factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; VCAM, vascular-cell adhesion molecule.

hours of *de novo* synthesis. Therefore, the mediators necessary for neutrophil adhesion are present early in the course of inflammation, whereas molecules leading to monocyte adhesion appear several hours later.

Why are monocytes subsequently recruited? A role for IL-6–sIL-6R α *trans* signaling in chemokine shift

In addition to adhesion molecules, the recruitment of leukocytes is dependent on the specificity of chemokines produced in the inflammatory site. IL-8 and monocyte chemotactic protein-1 (MCP-1) are the most important chemokines for the recruitment of PMN cells and monocytes, respectively. The transition from neutrophil to monocyte accumulation might be linked to these two chemokine production kinetics and functional properties. When neutrophils or other cells are stimulated by inflammatory cytokines, IL-8 is usually produced early and for 24 h, recruiting and activating more neutrophils locally [8,9]. Prolonged production of IL-8 on the contrary, might induce high concentrations of this chemokine in vessels, which inhibits both neutrophil adhesion to endothelium and extravasation [10]. MCP-1 production is usually delayed but sustained for several days; its accumulation does not desensitize cells and leads to late monocyte recruitment [8,9].

The transition from neutrophil to monocyte accumulation might also be secondary to a shift of the type of chemokine produced by stromal cells, inflammatory macrophages or neutrophils. Indeed, neutrophils stimulated with inflammatory cytokines for several hours selectively produced MCP-1 and not IL-8 [9]. In addition, it has been shown that IL-6 has a rather unexpected role in leukocyte recruitment in vivo, as a result of the fact that the IL-6–sIL-6R α complex can activate endothelial cells to secrete IL-8 and MCP-1, as well as the expression of adhesion molecules [11]. Noticeably, IL-6 activation of endothelial cells occurs in vivo, although these cells, like other stromal cells, express the gp130 transducing protein of the IL-6 receptor complex but not the 80 kDa ligand binding subunit, IL-6R α , and in vitro, this activation could only be achieved when IL-6 was combined with soluble recombinant IL-6R α . IL-6R α expression is limited in humans to leukocyte and hepatocyte membranes [12] but can be shed from the neutrophil membrane into a soluble form, which is found at high concentrations in neutrophil-enriched inflammatory fluids [13]. The sIL-6R α combines with IL-6 to bind gp130 on the membranes of stromal cells, and activates these cells in a mechanism called *trans* signaling, explaining the data obtained on endothelial cells [14]. Although the observation that IL-6-induced IL-8 secretion was reported by another group using supraphysiological sIL-6R α concentrations [15], other investigators have reported that the IL-6-sIL-6R α complex primarily induced MCP-1 and not IL-8 secretion by human mesangial cells, fibroblasts or blood mononuclear cells [16-18] (Table 1).

Recently, two groups including ours, made the observation that the IL-6-sIL-6R α complex favors the transition from neutrophil to monocyte in inflammation [19,20]. We used an *in vitro* model of thrombin-activated HUVECs (human umbilical vein endothelial cells), which have been shown to acquire an inflammatory phenotype characterized by IL-6, IL-8, MCP-1 secretion and adhesion molecule expression [21]. Addition of sIL-6R α , at concentrations comparable to those measured in inflammatory synovial fluids, to thrombinactivated HUVECs induced MCP-1 but not IL-8 secretion, which was even slightly inhibited [20]. Moreover, we observed that activation of neutrophils by IL-8 and other chemoattractants induces IL-6R α shedding, in a mechanism involving the TNF- α converting enzyme [22]. Interestingly, IL-6R α is expressed on monocyte membranes but its shedding is not induced by chemokine activation of these cells [12] (V. Marin et al., unpublished). In accordance with early observations in a model of C5-induced acute inflammation, monocyte recruitment in lungs was dependent on prior neutrophil recruitment [3]. These data suggest an interesting cascade: thrombin (or another proinflammatory agent) might activate endothelium (or other stromal cells) to produce IL-8 and other chemoattractants favoring Opinion



Fig. 1. IL-6–sIL-6Rα autocrine loop of endothelial activation mediates a chemokine shift and the transition from neutrophil to monocyte recruitment. Endothelial (or stromal cells) activation by proinflammatory molecules leads to PAF, IL-8 and IL-6 secretion, as well as leuko-endothelial adhesion molecule expression (blue arrows). Chemoattractants, originating from the endothelium or other cell sources, recruit neutrophils and induce IL-6Rα shedding from their membranes (red arrows). The combination of sIL-6Rα with IL-6 enables ligation to gp130 on endothelial-cell membrane (black arrows) and increase both IL-6 and MCP-1 endothelial (or stromal) cell secretion (green arrows) but not IL-8, favoring a transition from neutrophil to monocyte recruitment. Abbreviations: ICAM, intercellular adhesion molecule; IL, interleukin; MCP, monocyte chemotatcic protein; PAF, platelet activating factor; sIL-6R, soluble IL-6 receptor; VCAM, vascular-cell adhesion molecule.

early neutrophil recruitment in the inflammatory site. Activated PMN cells might release sIL- $6R\alpha$, which combines with locally produced IL-6, to activate endothelial cells to produce MCP-1 and not IL-8, decreasing neutrophil and favoring monocyte recruitment (Fig. 1). This could explain why in inflammatory animal models, the neutrophilic infiltrate is more dominant in IL-6 knockout mice than in wild-type animals [23]. Similar observations and conclusions have been made by Hurst et al. [19] using mesothelial cells and validated in IL-6 knockout mice. In wild-type animals, the local infiltrate of acute peritonitis was primarily made of neutrophils followed by monocytes, however, in the IL-6 knockout animals, neutrophils were the only cells present in the infiltrate. The injection of exogenous IL-6-sIL-6R α complex restored the monocytic influx [19].

Role of apoptosis in neutrophil fate at the inflammatory site

A transition from neutrophil to monocyte accumulation at the inflammatory site not only suggests a progression of events leading to monocyte recruitment but also the disappearance of neutrophils. Neutrophils are central cells in the defense of an organism against injury, notably infection, through their capacity to synthesise oxygen metabolites and to liberate various enzymes. However, these agents might also be toxic for normal surrounding tissues and potentially induce inflammatory diseases. Therefore, neutrophil functions must be rapidly negatively regulated. Numerous reports show that this is achieved through local death of aged PMN cells by apoptosis [24]. In addition, activation of PMN cells by phagocytosis of bacteria or more globally by phagocytosis mediated by Fc or complement receptors induces upregulation of programmed cell death genes and apoptosis [25]. Some cytokines and growth factors, such as granulocyte-colony-stimulating factor (G-CSF) prevent neutrophil death but interestingly, IL-6 has been shown to induce PMN-cell apoptosis [26]. More importantly, death by apoptosis induces loss of PMN-cell biological functions but prevents liberation of neutrophil intracellular toxic contents and further tissue injury [27].

Apoptosis induces the appearance of or changes of some molecules on leukocyte-cell membranes, notably negatively charged phosphatidylserine, sugars and ICAM-3. These molecules might be recognized by various receptors, such as the phosphatidylserine, the vitronectin, the scavenger receptors, or CD36 on macrophages leading to phagocytosis of dead cells [28]. In addition, CD44, the hyaluronan receptor, which is present on both neutrophil and macrophage surfaces also increases the phagocytosis of apoptotic neutrophils and has an important role in the resolution of



28



Fig. 2. Role of apoptotic neutrophil phagocytosis in the transition from neutrophil to monocyte recruitment. Apoptotic neutrophils expressing new membrane antigens (PS: phosphatidylserine, TSP: thrombospondin) are recognized by various receptors on macrophages (SCV Rec: scavenger receptors, PS Rec: phosphatidylserine receptor, α5β3: vitronectin receptor), leading to their phagocytosis. Phagocytosis of apoptotic PMN neutrophils by macrophages increases TGFβ and MCP-1 secretion and decreases IL-8 production (red arrows), leading to a chemokine shift favoring monocyte recruitment (green arrows). Abbreviations: ICAM, intercellular adhesion molecule; MCP, monocyte chemotactic protein; TGF, transforming growth factor; TNF, tumor necrosis factor.

inflammation [29,30]. For the phagocytosis process to be engaged, a previous persistent adhesive step mediated by homophilic CD31 interactions between apoptotic cells and phagocytes seems to be necessary, as recently reported [31]. A crucial aspect of this phenomenon is that in contrast to FCyR-mediated phagocytosis, phagocytosis of apoptotic cells decreases macrophage activation and cytokine production through secretion of transforming growth factor- β (TGF- β) [32,33]. Notably, IL-1 β , TNF- α and IL-8 are decreased. TGF-B also decreases the production of G-CSF by macrophages, thus favoring PMN-cell death [33]. However, contrary to other main proinflammatory cytokines, phagocytosis of apoptotic neutrophils induces MCP-1 production by macrophages [33]. Thus, PMN-cell apoptosis not only participates in neutrophil elimination but could also favor a chemokine shift leading to monocyte recruitment (Fig. 2).

Inflammatory macrophages emigrate to the draining lymph node

Following these different steps, neutrophils are depleted from the inflammatory site; by contrast, blood monocytes accumulate and differentiate into inflammatory macrophages, which complete phagocytosis and destruction of the injurious agents [1-3]. Contrary to neutrophils, monocytes and macrophages do not die locally but emigrate after several days in the local lymph nodes [34]. During this migratory process, monocytes differentiate into dendritic cells, upregulating HLA class II antigen membrane expression, and acquiring costimulatory molecules, such as CD80 and CD86 [35]. These cells might then present antigenic peptides to lymphocytes, contributing to the generation of an immune response.

Concluding remarks

To be beneficial, the inflammatory reaction must be acute, destroying the injurious agent in a timeand space-limited fashion, and inducing an immune response. This is achieved through a complex series of events characterized by local leukocyte recruitment, death and emigration [36]. The transition from neutrophil to monocyte recruitment participates in the efficient destruction of the noxious agent by the combined destructive and phagocytic actions of neutrophils and inflammatory macrophages. However, it also participates in the resolution of inflammation through elimination of neutrophils and the initiation of an immune response. We discussed here the role of a shift of locally produced chemokines and the potential orchestrating functions of IL-6 in this phenomenon. It remains probable, however, that other signals exist that promote this shift in chemokine production and leukocyte recruitment. Moreover, this adds a new function for IL-6; its role in inflammation remains to be elucidated. In numerous models of chronic inflammatory diseases, such as collagen-induced arthritis, murine colitis or experimental autoimmune encephalomyelitis, IL-6 is pro-inflammatory [37,38], whereas in models of acute inflammation (except in a recently reported model [39] of viral ocular inflammation), IL-6 is rather protective [23]. A way to reconcile these different aspects and an attractive hypothesis might be that in acute inflammation, IL-6 could have a rather

Opinion

protective role decreasing neutrophil and favoring monocyte recruitment leading to the resolution of inflammation and the initiation of an immune response. Alternatively, in chronic inflammation, as supported by experimental models of rheumatoid arthritis and Crohn's disease, IL-6 could have a rather detrimental role favoring mononuclear-cell accumulation at the site of injury, through continuous MCP-1 secretion, angioproliferation and anti-apoptotic functions on T cells [40]. Better knowledge of these mechanisms could help our understanding of how and why mediators of acute inflammation lead, in some circumstances, to chronic inflammation.

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