Published in final edited form as:

Thromb Haemost. 2014 May 5; 111(5): 1004–1006. doi:10.1160/TH13-09-0794.

Developmental endothelial locus-1 attenuates complement-dependent phagocytosis through inhibition of Mac-1-integrin

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Keywords

Del-1; Mac-1; iC3b; phagocytosis

Dear Editor

Integrins of the $\beta 2$ -family are exclusively expressed on hematopoietic cells and consist of a common $\beta 2$ -chain (CD18), which associates with one of four different alpha-chains, αL , αM , αX and αD (also designated CD11a, CD11b, CD11c and CD11d, respectively). Lymphocyte function-associated antigen (LFA)-1 ($\alpha L\beta 2$; CD11a/CD18) and macrophage-1 antigen (Mac-1) ($\alpha M\beta 2$; CR3; CD11b/CD18) are two prominent members of the $\beta 2$ -integrin family, interacting with immunoglobulin counter-receptors, such as intercellular adhesion molecule (ICAM)-1. Whereas the former is involved in leukocyte adhesion to the endothelium, hence inflammatory cell recruitment, and in immune synapse formation, Mac-1, also known as complement receptor-3 (CR3), plays important roles not only in leukocyte-endothelial interactions but also in complement-dependent opsonophagocytosis, via interaction with the complement fragment iC3b (1–6).

We have recently identified a novel function for Developmental endothelial locus-1 (Del-1), originally described as a matrix glycoprotein expressed and secreted by endothelial cells (1). Specifically, we showed that Del-1 functions as an antagonist of LFA-1-dependent inflammatory cell recruitment, competing with ICAM-1 for LFA-1, thereby acting as an

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Conflict of interest

The authors declare no conflict of interest.

endogenous inhibitor of leukocyte adhesion to endothelial cells (7, 8). Given that Del-1 is a ligand of LFA-1 (7) and that LFA-1 and Mac-1 share several common ligands (5), we set out to investigate, whether Del-1 could also interact with Mac-1 and could modulate its function.

This hypothesis was initially tested using a solid phase binding assay. Del-1 bound to immobilized purified Mac-1 in a concentration-dependent manner (figure 1A). To verify that this protein-protein interaction could be relevant for cell function, we tested the adhesion of CHO cells expressing Mac-1 (CHO-Mac-1) to immobilized Del-1. Compared to CHO cells transfected with the neomycin resistance vector alone (CHO-Neo), CHO-Mac-1 cells displayed increased adhesion to Del-1 (figure 1B). We next studied the adhesion of RAW 264.7 macrophages to immobilized Del-1. RAW 264.7 macrophages exhibited increased adhesion to immobilized Del-1, as compared to control immobilized protein, whereas pre-treatment of the cells with inhibitory anti-CD11b or anti-CD11a antibodies resulted in a significant reduction of adhesion to Del-1 (figure 1C), thus suggesting that, besides LFA-1, also Mac-1 interacts with Del-1. To conclusively confirm the role of Mac-1 in macrophage binding to Del-1, we assessed the adhesion of bone marrow-derived macrophages isolated from wild type or CD11b $-/-$ mice to immobilized Del-1. Consistent with the findings presented above, the adhesion of CD11b $-/-$ macrophages to immobilized Del-1 was significantly impaired, as compared to wild-type controls (figure 1D).

A variety of Mac-1 ligands are known. These include fibrinogen, RAGE, ICAM-1 and iC3b (1–4). However, phagocytosis of complement opsonized particles is considered an important, if not the major, function of Mac-1, and this phagocytic function has been recently implicated in autoimmune disease pathogenesis (9). To address whether Del-1 interferes with the binding of Mac-1 to iC3b, we used a static adhesion assay involving CHO-Mac-1 cells. Pre-treatment of cells with soluble Del-1 significantly decreased the adhesion of CHO-Mac-1 cells to iC3b (figure 1E, supplementary figure 1). The specificity of the inhibitory action of Del-1 was proven by heat-denaturation of Del-1. This pretreatment of Del-1 abrogated its inhibitory effect on Mac-1-dependent adhesion to iC3b (supplementary figure 1). We further asked whether the aforementioned inhibitory effect of Del-1 could influence complement-dependent Mac-1-mediated phagocytosis. For this reason, a phagocytosis assay using iC3b-coated red blood cells (C3bi-RBCs) was conducted. Although pretreatment of RAW 264.7 macrophages with Del-1 did not significantly affect the number of C3bi-RBCs bound to RAW 264.7 macrophages (figure 1F), it significantly reduced the percentage of RAW 264.7 macrophages that engulfed C3bi-RBCs (figure 1G and supplementary figure 2).

In summary, our present study expands the regulatory role of Del-1 to include the inhibition of complement-dependent phagocytosis in macrophages. Specifically, Del-1 inhibits phagocytosis of iC3b-coated particles by antagonizing Mac-1 binding to iC3b. We have previously reported that Del-1 mediates its inhibitory effect on neutrophil recruitment in the course of inflammation via antagonizing LFA-1-dependent adhesion. The ability of Del-1 to interact with both Mac-1 and LFA-1 are in line with our previous report that LFA-1-deficiency could not completely abolish the interaction of neutrophils with Del-1 (7). Moreover, antibody-mediated inhibition of Mac-1 integrin resulted in a further reduction of

adhesion of LFA-1-deficient neutrophils to Del-1 (7). We therefore propose that the relative abundance and functional role of LFA-1 and Mac-1 in different hematopoietic cell populations could determine which of these two integrins is targeted by Del-1 in distinct leukocyte functions pertinent to inflammation.

Several lines of evidence suggest a key role for Mac-1 in disease pathogenesis. A genetic variation in the coding region of the ITGAM gene (encoding the CD11b subunit of Mac-1), which results in a single amino acid substitution of the extracellular domain of CD11b (rs1143679, conversion of arginine to histidine at position 77; R77H), has been associated with the development of systemic lupus erythematosus (10). It is thought that the main defect in leukocytes derived from individuals with these genetic variants is reduced Mac-1-mediated phagocytosis of iC3b opsonized particles, suggesting an important role for Mac-1-mediated phagocytosis in preserving self-tolerance (9). On the other hand, Mac-1-dependent leukocyte activation has been shown to mediate tissue injury in several experimental settings (11–13). Finally, Mac-1 acts as an important receptor for phagocytosis, thereby promoting bacterial clearance (14). However, several studies suggest that certain pathogens may exploit Mac-1 for safe entry within macrophages (15). It is thus conceivable that the proper regulation of Mac-1 function is essential for the maintenance of tissue homeostasis as well as of defense against invading pathogens. We here propose that Del-1 acts as a regulator of Mac-1-function and in particular of Mac-1-mediated complement-dependent phagocytosis. This regulatory role of Del-1 is consistent with the proposed function of Del-1 as an endogenous homeostatic agent contributing to tissue homeostasis as it may have important implications in the course of inflammatory or infectious diseases and tissue injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants of the Deutsche Forschungsgemeinschaft (T.C.), from National Institutes of Health (DE021685 to G.H.) and from the National Research Foundation of Korea (NRF), Ministry of Education, Science and Technology of Korea (2011-0014447 and 2011-0031547 to E.Y.C.).

References

1. Chavakis E, Choi EY, Chavakis T. Novel aspects in the regulation of the leukocyte adhesion cascade. *Thromb Haemost.* 2009; 102:191–197. [PubMed: 19652868]
2. Gahmberg CG, Fagerholm SC, Nurmi SM, et al. Regulation of integrin activity and signalling. *Biochim Biophys Acta.* 2009; 1790:431–444. [PubMed: 19289150]
3. Hajishengallis G, Chavakis T. Endogenous modulators of inflammatory cell recruitment. *Trends Immunol.* 2013; 34:1–6. [PubMed: 22951309]
4. Lim J, Wiedemann A, Tzircotis G, et al. An essential role for talin during alpha(M)beta(2)-mediated phagocytosis. *Mol Biol Cell.* 2007; 18:976–985. [PubMed: 17202407]
5. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. *Annu Rev Immunol.* 2007; 25:619–647. [PubMed: 17201681]
6. Phillipson M, Heit B, Colarusso P, et al. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med.* 2006; 203:2569–2575. [PubMed: 17116736]

7. Choi EY, Chavakis E, Czabanka MA, et al. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. *Science*. 2008; 322:1101–1104. [PubMed: 19008446]
8. Eskin MA, Jotwani R, Abe T, et al. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nat Immunol*. 2012; 13:465–473. [PubMed: 22447028]
9. Fossati-Jimack L, Ling GS, Cortini A, et al. Phagocytosis is the main CR3-mediated function affected by the lupus-associated variant of CD11b in human myeloid cells. *PLoS One*. 2013; 8:e57082. [PubMed: 23451151]
10. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin- α (M) (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat Genet*. 2008; 40:152–154. [PubMed: 18204448]
11. Hirahashi J, Hishikawa K, Kaname S, et al. Mac-1 (CD11b/CD18) links inflammation and thrombosis after glomerular injury. *Circulation*. 2009; 120:1255–1265. [PubMed: 19752320]
12. Shimizu K, Libby P, Shubiki R, et al. Leukocyte integrin Mac-1 promotes acute cardiac allograft rejection. *Circulation*. 2008; 117:1997–2008. [PubMed: 18378617]
13. Wang Y, Sakuma M, Chen Z, et al. Leukocyte engagement of platelet glycoprotein Iba1 via the integrin Mac-1 is critical for the biological response to vascular injury. *Circulation*. 2005; 112:2993–3000. [PubMed: 16260637]
14. Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science*. 1998; 282:1717–1721. [PubMed: 9831565]
15. Hajishengallis G, Lambris JD. Microbial manipulation of receptor crosstalk in innate immunity. *Nat Rev Immunol*. 2011; 11:187–200. [PubMed: 21350579]

Figure 1. Del-1 interferes with Mac-1-mediated phagocytosis of iC3b coated RBCs

A. Binding of Del-1 to immobilized Mac-1, using a solid phase adhesion assay. One representative experiment is shown. Similar results were observed in three independent experiments. **B.** Adhesion of CHO cells expressing recombinant Mac-1 (CHO-Mac-1) or control-transfected CHO cells (CHO-neo) to immobilized BSA or Del-1. Data derived from four independent experiments. **C.** Adhesion of RAW 264.7 cells to Del-1 is impaired by anti-CD11b or anti-CD11a antibodies. Cells treated with control antibody served as the control (ctrl). Data derived from three independent experiments. **D.** Adhesion of bone marrow derived macrophages from wild type and CD11b-deficient mice to Del-1. Macrophages derived from three and four mice from each group, respectively, were used. **E.** Relative adhesion of CHO-Mac-1 cells pretreated with Del-1 (10μg/mL) or with BSA to immobilized iC3b is shown. Data derived from five independent experiments are shown. **F.** RAW 264.7 macrophages were challenged with C3bi-RBCs. Macrophages were pretreated with BSA, Del-1 (10μg/mL) or an anti-CD11b antibody or left untreated (ctrl) before the addition of C3bi-RBCs, as indicated. Binding of C3bi-RBCs to RAW 264.7 macrophages is shown. Data presented as the number of bound C3bi-RBCs per one hundred RAW 264.7 macrophages. **G.** Percentage of RAW 264.7 macrophages that have phagocytosed one to three C3bi-RBCs. N=5 for each group in F and G. Data derived from one representative experiment are shown. Similar results were obtained in at least two more independent experiments demonstrating the inhibitory role of Del-1 in phagocytosis of C3bi-RBC. Statistical analysis was performed compared to cells treated with BSA. Data presented as mean ± SD. *p < 0.05, ** p < 0.01.