Sem comunidade   WoS

2012

M-ficolin and leukosialin (CD43): new partners in neutrophil adhesion

M-ficolin and leukosialin (CD43): new partners in neutrophil adhesion

Andrea N. Moreno-Amaral,*,† Evelyne Gout,‡ Claudia Danella-Polli,*,§ Fanny Tabarin,*
Philippe Lesavre,* Gabriela Pereira-da-Silva,§ Nicole M. Thielens,‡
and Lise Halbwachs-Mecarelli*,1

*Institut National de la Santé et de la Recherche Medicale INSERM U845, Université Paris Descartes, Sorbonne Paris Cité, Hôpital Necker, Paris, France; †Group for Advanced Molecular Investigation, Center for Health and Biological Sciences, Pontifical Catholic University of Paraná, Curitiba, PR, Brazil; ‡Institut de Biologie Structurale Jean-Pierre Ebel, UMR 5075 Centre National de la Recherche Scientifique, Commissariat à l'Energie Atomique, Université Joseph Fourier-Grenoble, Grenoble, France; and §Department of Maternal-Infant Nursing and Public Health, University of São Paulo at Ribeirão Preto College of Nursing, São Paulo, Brazil

ABSTRACT

M-ficolin specificity for sialylated ligands prompted us to investigate its interactions with the main membrane sialoprotein of human neutrophils, CD43. rM-ficolin bound CD43 and prevented the access of anti-CD43 mAb. Moreover, rM-ficolin reacted exclusively with CD43 on Western blots of neutrophil lysate. We confirmed that M-ficolin is secreted by fMLP-activated neutrophils, and this endogenous M-ficolin also binds to CD43 and competes with anti-CD43 mAb. Anti-CD43 antibody crosslinking or fMLP resulted in M-ficolin and CD43 colocalization on polarized neutrophils. The binding of rM-ficolin to resting neutrophils induced cell polarization, adhesion, and homotypic aggregation as anti-CD43 mAb. The M-ficolin Y271F mutant, unable to bind sialic acid, neither reacted with neutrophils nor modulated their functions. Finally, rM-ficolin activated the lectin complement pathway on neutrophils. These results emphasize a new function of M-ficolin, different from ficolin pathogen recognition, i.e., a participation to neutrophil adhesion potentially important in early inflammation, as nanomolar agonist concentrations are sufficient to mobilize M-ficolin to the neutrophil surface. This multivalent lectin could then endow the antiadhesive CD43, essentially designed to prevent leukocyte aggregation in the blood flow, with new adhesive properties and explain, at least in part, dual-adhesive/antiadhesive roles of CD43 in neutrophil recruitment. *J. Leukoc. Biol.* **91: 469–474; 2012.**

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

Introduction

Ficolins are pattern-recognition molecules that interact with micro-organism surfaces and activate complement. A peculiarity of human M-ficolin (Ficolin-1) and rodent Ficolin B is their ability to bind, via their PAMP recognition domain, to sialylated ligands [1–6]. Although sialic acid is found on some pathogens, it mainly decorates self-cells, and human M-ficolin has been shown to bind sialylated ligands on human monocytes and neutrophils [2, 7, 8]. The specificity of M-ficolin for sialylated ligands is thus puzzling and suggests that its functions are not restricted to antimicrobial defense [9–11].

M-ficolin has been identified in monocytes and in easily mobilizable granules of neutrophils [2, 3, 7, 12]. We here investigated the response of human neutrophils to M-ficolin binding on their surface and the molecular basis of this binding, with special emphasis on leukosialin (CD43), a major membrane sialoglycoprotein related to leukocyte adhesion, aggregation, and migration. CD43 has been described so far as a ligand of endothelial E-selectin [13, 14], but the role of E-selectin interactions with CD43 in leukocyte recruitment is still a matter of controversy [15, 16]. M-ficolin localization in neutrophils and sugar specificity made it a likely candidate for a new CD43-binding molecule.

MATERIALS AND METHODS

Neutrophils

Blood was obtained from the French blood transfusion center Etablissement Français du Sang (Paris, France) with agreement for studies on healthy volunteers. Neutrophils were isolated from EDTA-anticoagulated blood after platelet depletion by centrifugation on polymorphprep (Axis-Shield, Oslo, Norway), as described [17]. Isolated neutrophils were resuspended in TBS or KRPG-BSA containing 1 mM Ca^{2+}/Mg^{2+}, as mentioned below.

1. Correspondence: INSERM U845, Batiment Lavoisier, Hôpital Necker, 149 rue de Sevres, 75015 Paris, France. E-mail: lise.halbwachs@inserm.fr

M-ficolin

rM-ficolin and Y271F mutant [4] were labeled with EZ-Link Sulfo-NHS-LC-biotin (ref. 21,327, Thermo Scientific, Rockford, IL, USA), according to the manufacturer's instructions. Briefly, 0.1 mg recombinant protein (1.2 mg/ml in Hepes 20 mM, pH 7.5, NaCl 0.15 M, Ca^{++} 5 mM) was incubated in a microtube containing 1 mg Sulfo-NHS-LC-biotin. The excess biotin was then removed using a desalting column (Zeba spin column, ref. 89,882, Thermo Scientific) equilibrated in TBS. Biotinylated proteins were kept for up to 1 month in TBS with 0.1% sodium azide at 4°C.

Determination of membrane-bound M-ficolin or membrane markers by flow cytometry

Neutrophils (10^6/ml) were incubated in TBS with rM-ficolin for 5 min at 4°C to measure the binding of exogenous M-ficolin or in KRPG-BSA with 10^{-8} M fMLP for 15 min at 37°C to analyze the endogenous M-ficolin. Cells were then labeled and analyzed by flow cytometry [18]. Biotinylated rM-ficolin was revealed with PE-streptavidin (Beckman Coulter, Brea, CA, USA), whereas endogenous M-ficolin was labeled with a rabbit anti-human M-ficolin antibody (Hycult Biotech, Plymouth Meeting, PA, USA) and a secondary goat anti-rabbit IgG labeled with Alexa-555 (Molecular Probes, Eugene, OR, USA). The level of membrane CD43 was measured similarly after labeling with G10 anti-CD43 mAb (BD PharMingen, San Diego, CA, USA) and PE-labeled anti-mouse IgG (Beckman Coulter).

Analysis of M-ficolin or CD43 membrane localization

Neutrophils in KRPG-BSA were incubated without or with biotinyl-rM-ficolin or fMLP for 10 min at 37°C or with a specific FITC-labeled anti-CD43 mAb (G10, BD PharMingen) and a F(ab)'2 anti-mouse IgG, to cross-link CD43 [19]. Cells were fixed with 3.7% PFA and 0.5% glutaraldehyde before labeling and analysis by fluorescence microscopy after cytocentrifugation.

Western blot

Neutrophils were lysed in PBS, 1% Nonidet P-40, with protease inhibitors [aprotinin (0.1 trypsin inhibitor unit/ml), leupeptin (100 μg/ml), chymostatin (20 μg/ml), and PMSF (1 mM; Sigma Chemical Co., St. Louis, MO, USA)]. After a rapid centrifugation at 11,000 g to remove nuclei, each lysate was supplemented with one-half of its volume of 2× Laemmli SDS-reducing sample buffer and analyzed after SDS-PAGE 7.5% by Western blotting. Western blots, saturated overnight with 3% BSA in TBST, were incubated with 1 μg/ml biotin-labeled rM-ficolin for 30 min at 4°C, followed by HRP-extravidin (E2886, Sigma Chemical Co.). One part of each blot was saturated separately with 5% nonfat dry milk in TBST and treated overnight at 4°C with G10 anti-CD43 mAb and then with HRP-anti-mouse IgG (GE Healthcare, Waukesha, WI, USA). Western blots were revealed by chemiluminescence (SuperSignal West Pico substrate, Thermo Scientific).

Cell adhesion and homotypic aggregation

Neutrophils in KRPG-BSA buffer, in a 96-well plate coated with 1% gelatin, were incubated with rM-ficolin for 15 min at 37°C and then, when mentioned with TNF-α. Washed adherent cells were fixed for 10 min with 3.7% PFA, stained with 0.2% crystal violet, and lysed with 2% SDS and the absorbance measured at 550 nm. To measure aggregation, neutrophil suspension (10^6/ml in KRPG-BSA), without or with rM-ficolin, was aliquoted into 1.5 ml Eppendorf tubes containing a magnet (6 mm×1 mm) and was stirred at a low speed in a 37°C water bath for 0–30 min. Cells were then fixed with 2% glutaraldehyde and applied to poly-L-lysine-coated coverslips before analysis by optical microscopy.

Complement activation

Neutrophils (10^6/ml in TBS with 1 mM Ca^{++} and Mg^{++} and 1% BSA), treated or not with rM-ficolin for 15 min at room temperature, were incubated with 1/10 final dilutions of NHS or B- or C2-depleted serum (Quidel, San Diego, CA, USA) as a source of complement. After a further incubation for 30 min at 37°C, neutrophils were washed three times with PBS–1% BSA–0.1% sodium azide and labeled with an anti-C3d mAb (Quidel) and a secondary PE-labeled goat F(ab')$_2$ anti-mouse IgG antibody (Beckman Coulter). The level of cell-bound C3 was measured by flow cytometry.

RESULTS AND DISCUSSION

We first confirmed previously published data showing that exogenous rM-ficolin binds to sialic acid on neutrophils [8]. Indeed, the interaction with neutrophils is inhibited by GlcNAc (**Fig. 1A**), is calcium-dependent (Fig. 1B), and is not observed with rM-ficolin containing the Y271F mutation, which abolishes M-ficolin binding to sialic acid [4] (Fig. 1C). In our experimental setting, M-ficolin was able to bind to neutrophils in
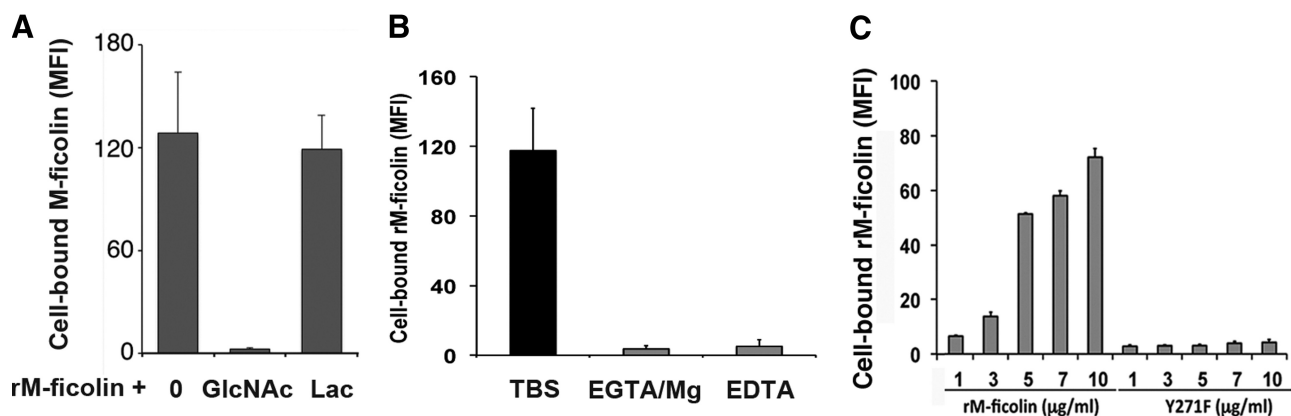

Figure 1. Binding of exogenous rM-ficolin to the neutrophil surface. Neutrophils were incubated for 5 min at 4°C with 5 μg/ml biotinylated rM-ficolin in the presence or not of 50 mM GlcNAc or lactose (Lac; A) and of 10 mM EGTA with 5 mM Mg or of 5 mM EDTA (B). (C) Neutrophils were incubated with increasing concentrations of biotinylated rM-ficolin or the Y271F mutant. (A–C) Neutrophils were then treated with PE-labeled streptavidin, and the presence of cell-bound rM-ficolin was measured by flow cytometry. Results are expressed as mean fluorescence intensity (MFI) ± SD (n=3 experiments).

TBS without added calcium, but the complete inhibition of this binding by EDTA or EGTA-Mg (Fig. 1B) implied that metallic ions, such as Ca, were required. Those are most probably provided by neutrophils themselves, as membrane-associated Ca^{++} [20, 21] and slow calcium efflux [22, 23] have been described in resting neutrophils.

Observations from the literature point out CD43 as a leading candidate for the M-ficolin ligand on neutrophils: 1) specific radiolabeling of sialic acids identified CD43 as the predominant membrane sialoprotein of neutrophil [24]; and 2) M-ficolin binds monocytes, neutrophils, and activated but not nonactivated T lymphocytes [8]. This is reminiscent of differences in CD43 O-glycosylation among leukocytes. Indeed, on resting T-lymphocytes, CD43 contains the tetrasaccharide NeuAc $(\alpha2–3)$Gal$(\beta1–3)$[NeuAc$(\alpha2–6)$]GalNAc, whereas on activated T lymphocytes and neutrophils, it contains the branched hexasaccharide NeuAc$(\alpha2–3)$Gal$(\beta1–3)$[NeuAc$(\alpha2–3)$Gal$(\beta1–4)$GlcNAc$(\beta1–6)$]GalNAc [25, 26]. Interestingly, only the latter saccharide was identified as a ligand of M-ficolin by glycoarray screening (Supplemental Table 1) [4].

Our observation that rM-ficolin interaction with neutrophils prevented the anti-CD43 mAb binding (G10 mAb as in **Fig. 2A** or L60 mAb; data not shown) supported our hypothesis of CD43/M-ficolin interactions. It indeed revealed a steric hindrance of CD43 epitopes by cell-bound M-ficolin in a specific way, as no interference was observed with antibodies reacting with membrane glycoproteins CD66, CD44, P-selectin glycoprotein ligand 1 (CD162), or CD11b (data not shown). The anti-CD43 mAb, on the other hand, did not inhibit the binding of M-ficolin. This is not surprising, as it binds a single epitope on the CD43 molecule, which presents numerous sialylated ligands along its mucin-like structure.

The final demonstration that CD43 is the major M-ficolin ligand on neutrophils was given by Western blot analysis of a total neutrophil lysate. After incubation with biotinylated rM-ficolin, Western blots revealed a restricted specificity of M-ficolin for CD43 (Fig. 2B). The interaction of rM-ficolin with CD43 was inhibited by GlcNAc (Fig. 2A and B) and did not occur with the Y271F M-ficolin mutant, devoid of sialic acid-binding ability (Fig. 2A). rM-ficolin thus reacts with neutrophil CD43 via its sialic acid-binding site.

We had shown that CD43 cross-linking by mAb induces a capping of CD43 in the uropod of polarized neutrophils [19, 27]. We used this characteristic to see if neutrophils express other M-ficolin ligands than CD43. When CD43 capping was induced by anti-CD43 antibody cross-linking, before the addition of rM-ficolin, we observed a colocalization of the cell-bound rM-ficolin with CD43 (Fig. 2C). As reported previously [7], neutrophil secretory granules contain pools of M-ficolin, which are secreted upon stimulation and bind to the plasma membrane. We confirmed that resting neutrophils, isolated from EDTA-anticoagulated blood, do not express membrane M-ficolin [7], but stimulation by 10^{-8} M fMLP, 10 ng/ml TNF, or 25 ng/ml IL-8 results in the appearance of M-ficolin on the neutrophil surface (data not shown). The level of cell-bound endogenous M-ficolin, observed after neutrophil incubation with 10 nM fMLP, was in the same range as that observed on resting cells incubated with 3 μg/ml rM-ficolin (**Fig. 3A**). The fMLP-induced activation, which releases intracellular M-ficolin, resulted in a decrease of anti-CD43 neutrophil labeling, completely prevented by GlcNAc (Fig. 3B), thus showing that the endogenous M-ficolin also interacts with membrane CD43. The CD43 steric hindrance by exogenous rM-ficolin was not so striking as by exogenous rM-ficolin. This is most
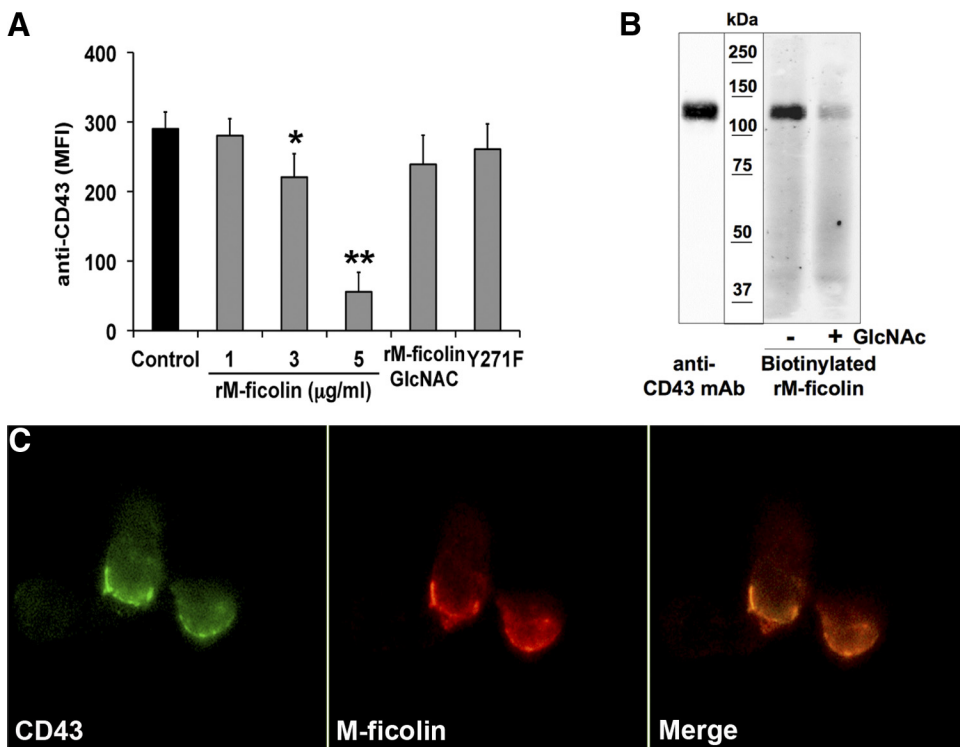

Figure 2. Exogenous rM-ficolin interacts with CD43. (A) Neutrophils were labeled with the G10 anti-CD43 mAb after incubation for 5 min at 4°C with increasing concentrations of rM-ficolin, with 5 μg/ml rM-ficolin in the presence of 50 mM GlcNAc, or with the 5 μg/ml Y271F mutant. CD43 labeling was analyzed by flow cytometry and results expressed as mean ± SD (n=3 experiments). *$P <$ 0.05; **$P <$ 0.01, by Student's t test. (B) Neutrophil lysate was analyzed by Western blotting, revealed with the anti-CD43 mAb (left) or with 1 μg/ml biotinylated rM-ficolin. (C) Neutrophils were incubated at 4°C for 30 min with an FITC-labeled anti-CD43 mAb (green) and F(ab')$_2$ anti-mouse IgG, then at 37°C to induce CD43 capping, and fixed with PFA/glutaraldehyde, as described in Materials and Methods. They were finally labeled with biotinyl-M-ficolin and Alexa-555 streptavidin (red) and analyzed by fluorescence microscopy.

A

Cell-bound M-ficolin

B

anti-CD43 (MFI)

C

Figure 3. Endogenous M-ficolin is secreted by stimulated neutrophils and interacts with membrane CD43. (A) Endogenous and rM-ficolin were measured by flow cytometry using the same rabbit anti-M-ficolin antibody and an Alexa-555-labeled secondary antibody. (B) Neutrophils were labeled with anti-CD43 G10 mAb after a 5-min incubation at 37°C with 10^{-8} M fMLP, without or with 50 mM GlcNAc. *$P < 0.05$. (C) Neutrophils were incubated at 37°C with fMLP (10^{-8} M), fixed with PFA/glutaraldehyde, then labeled with the rabbit anti-M-ficolin antibody and an Alexa-555-labeled secondary antibody, and analyzed as in Fig. 2C.
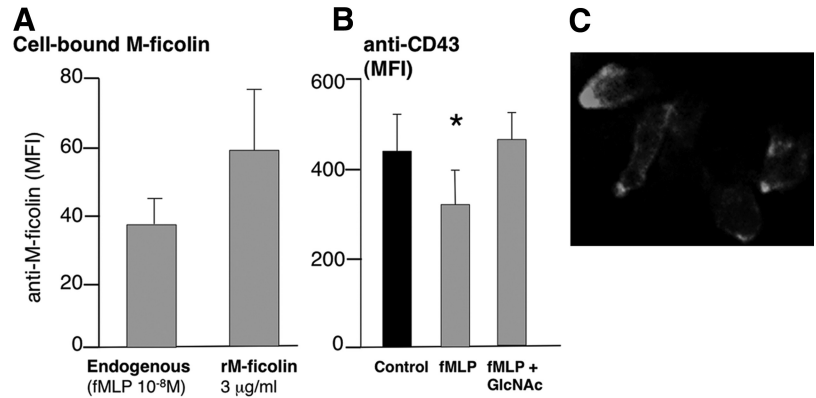
probably related to the N-terminal localization of anti-CD43 mAb epitopes in a zone emerging from the cell glycocalyx as a result of the extended structure of the molecule. This would be more accessible to exogenous M-ficolin than to the secreted M-ficolin, which coming from inside the cell, would preferentially bind carbohydrates immediately proximal to the membrane.

One should point out that the decreased anti-CD43 labeling, resulting from M-ficolin-covering CD43 epitopes, differs from the important proteolysis-mediated down-regulation of CD43, observed with stronger neutrophil activation [28].

M-ficolin membrane distribution also argued for CD43 as the major ligand of endogenous M-ficolin. Indeed, stimulation by fMLP is known to result in neutrophil polarization with a clustering of membrane CD43 in the cell uropod [19, 27]. A similar, striking redistribution of membrane-bound endogenous M-ficolin was observed in the uropod of fMLP-activated neutrophils (Fig. 3C).
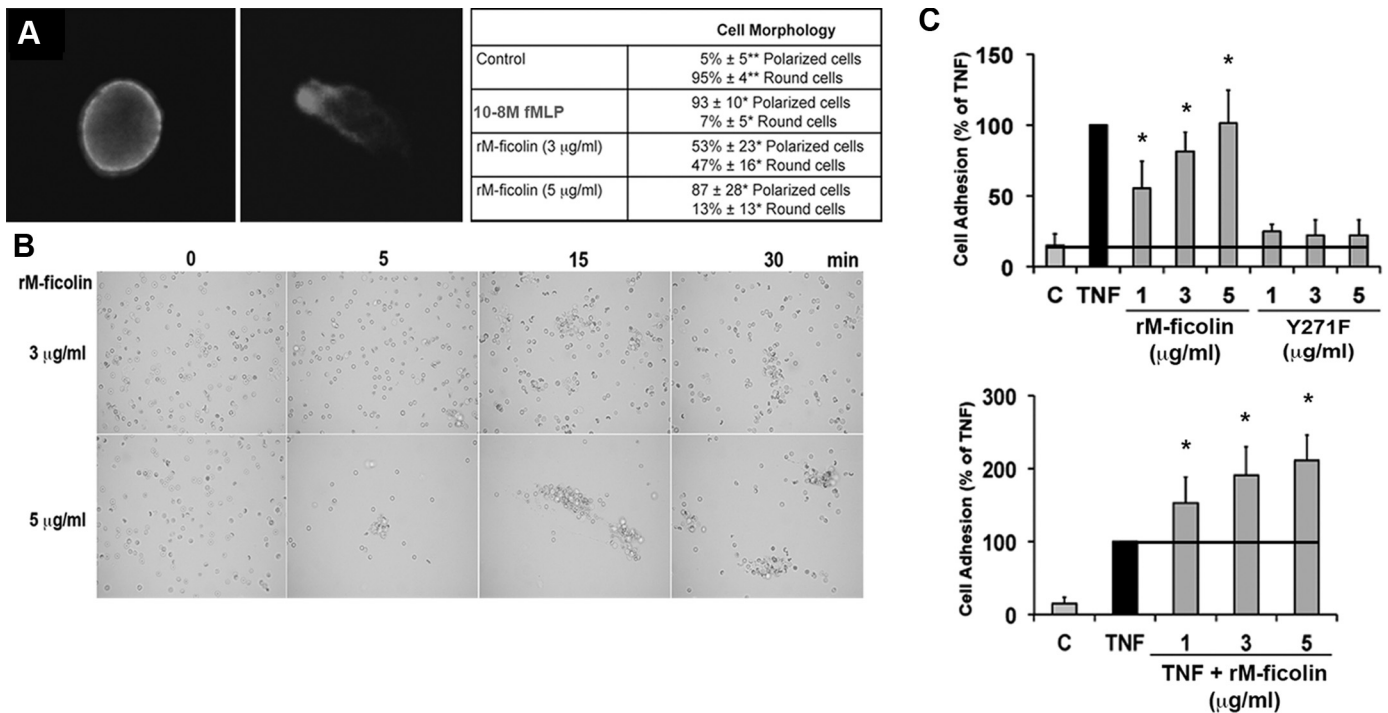
A

	Cell Morphology
Control	5% ± 5** Polarized cells 95% ± 4** Round cells
10-8M fMLP	93 ± 10* Polarized cells 7% ± 5* Round cells
rM-ficolin (3 µg/ml)	53% ± 23* Polarized cells 47% ± 16* Round cells
rM-ficolin (5 µg/ml)	87 ± 28* Polarized cells 13% ± 13* Round cells

B

C

Figure 4. Cell-bound M-ficolin promotes neutrophil polarization, adhesion, and complement activation. (A) Neutrophils were treated for 5 min at 4°C with 5 µg/ml biotinylated rM-ficolin and kept at 4°C (left) or incubated at 37°C for 10 min (middle) and fixed with PFA/glutaraldehyde. After labeling with Alexa-555 streptavidin, cells were cytocentrifuged, visualized by fluorescence microscopy with a 60× objective, and the cell morphology analyzed (right table; $n=3$). (B) In the aggregation assay, neutrophils were incubated with rM-ficolin (5 µg/ml) for 0–30 min at 37°C. Images were obtained by optical microscopy (40× objective). (C) For the adhesion assay, neutrophils were incubated without or with rM-ficolin or its Y271F mutant for 15 min at 37°C in a 96-well, gelatin-coated plate (upper panel). Cells incubated with TNF (10 ng/ml) were used as positive control. In the lower panel, neutrophils were incubated with different concentrations of rM-ficolin for 15 min at 37°C and then with TNF (10 ng/ml) for 30 min at 37°C. Results are expressed as percent of the crystal violet absorption obtained with TNF alone (mean±SD; $n=3$ experiments). *$P < 0.05$, by Student's t test.
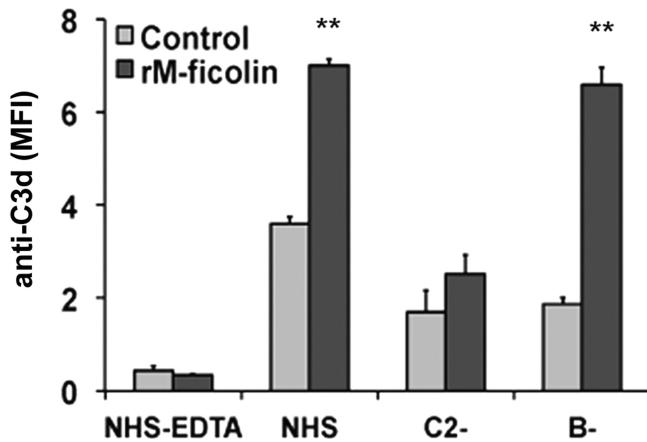
C3 deposition

Figure 5. Cell-bound M-ficolin activates the lectin complement pathway. Neutrophils were treated or not with rM-ficolin (5 μg/ml) for 15 min at room temperature and then with 1/10 dilutions of NHS in the presence or absence of 10 mM EDTA, B-deficient (B⁻), or C2-depleted (C2⁻) serum and incubated further for 30 min at 37°C. The level of neutrophil-bound C3 was measured by flow cytometry with an anti-C3d mAb and a PE-labeled secondary antibody. Results are expressed as mean ± SD (n=3). **$P < 0.01$, by Student's t test.

CD43 participation to neutrophil adhesion, migration, and activation has been shown in vitro, using anti-CD43 mAb cross-linking and assuming that this mimicked the effect of an unknown, multimeric, CD43-binding molecule [27, 29, 30]. M-ficolin could fulfill this function, as it is an oligomeric protein endowed with multivalent binding sites for sialylated ligands. If M-ficolin is able to cross-link cell-surface CD43 molecules, it should trigger cell functions described with anti-CD43 mAb. Indeed, the binding of exogenous rM-ficolin to unactivated neutrophils resulted by itself, at 37°C in the absence of other neutrophil-activating stimuli, in the cell polarization and a redistribution of rM-ficolin in the uropod (**Fig. 4A**). This polarization most probably initiates neutrophil locomotion by analogy with the effect of anti-CD43 cross-linking [27]. Moreover, exposure to rM-ficolin triggered neutrophil homotypic aggregation (Fig. 4B) and neutrophil adhesion to gelatin and amplified the adhesion induced by TNF-α (Fig. 4C). None of these effects were observed with the Y271F mutant of M-ficolin.

Finally, M-ficolin initiates the complement lectin pathway, via MASP-1 and MASP-2 activation [1, 3]. We here show that the binding of exogenous rM-ficolin activates complement on the neutrophil surface, resulting in membrane C3d deposition (**Fig. 5**). The rM-ficolin-dependent increase in C3d deposition occurred presumably via the lectin pathway, as it was inhibited in C2-depleted but not in factor B-depleted serum. We had previously reported an activation of the complement alternative pathway on cytokine- or serum (C5a)-stimulated neutrophils [18]. The inhibition of C3 deposition on neutrophils incubated in C2-depleted serum, even in the absence of exogenous rM-ficolin (Fig. 5, light, gray bars), suggests a participation of the lectin pathway, presumably triggered by

the endogenous M-ficolin, secreted by C5a-stimulated neutrophils. We have shown previously that complement activation on the cell surface amplifies neutrophil proinflammatory responses [18].

These data bring novel insights into the putative functions of M-ficolin and CD43. M-ficolin is presented for the first time as a partner of neutrophil adhesion and migration. On the other hand, the repulsive CD43 molecule here gains new, adhesive properties by covering its negatively charged sialic acids with a multivalent lectin able to interact with various acetylated ligands. This could explain some of the data showing a proadhesive role of CD43 in neutrophil recruitment [31, 32].

AUTHORSHIP

A.N.M-A., N.M.T., and L.H-M. designed experiments. A.N.M-A., C.D-P., and F.T. performed experiments. E.G. prepared WT and mutant rM-ficolins. A.N.M-A., P.L., G.P-S., N.M.T., and L.H-M. analyzed data and corrected the manuscript, which was written by L.H-M. and A.N.M-A.

ACKNOWLEDGMENTS

REFERENCES

1. Frederiksen, P. D., Thiel, S., Larsen, C. B., Jensenius, J. C. (2005) M-ficolin, an innate immune defence molecule, binds patterns of acetyl groups and activates complement. *Scand. J. Immunol.* **62,** 462–473.
2. Teh, C., Le, Y., Lee, S. H., Lu, J. (2000) M-ficolin is expressed on monocytes and is a lectin binding to *N*-acetyl-D-glucosamine and mediates monocyte adhesion and phagocytosis of *Escherichia coli. Immunology* **101,** 225–232.
3. Liu, Y., Endo, Y., Iwaki, D., Nakata, M., Matsushita, M., Wada, I., Inoue, K., Munakata, M., Fujita, T. (2005) Human M-ficolin is a secretory protein that activates the lectin complement pathway. *J. Immunol.* **175,** 3150–3156.
4. Gout, E., Garlatti, V., Smith, D. F., Lacroix, M., Dumestre-Perard, C., Lunardi, T., Martin, L., Cesbron, J. Y., Arlaud, G. J., Gaboriaud, C., Thielens, N. M. (2010) Carbohydrate recognition properties of human ficolins: glycan array screening reveals the sialic acid binding specificity of M-ficolin. *J. Biol. Chem.* **285,** 6612–6622.
5. Endo, Y., Nakazawa, N., Liu, Y., Iwaki, D., Takahashi, M., Fujita, T., Nakata, M., Matsushita, M. (2005) Carbohydrate-binding specificities of mouse ficolin A, a splicing variant of ficolin A and ficolin B and their complex formation with MASP-2 and sMAP. *Immunogenetics* **57,** 837–844.
6. Girija, U. V., Mitchell, D. A., Roscher, S., Wallis, R. (2011) Carbohydrate recognition and complement activation by rat ficolin-B. *Eur. J. Immunol.* **41,** 214–223.
7. Rorvig, S., Honore, C., Larsson, L. I., Ohlsson, S., Pedersen, C. C., Jacobsen, L. C., Cowland, J. B., Garred, P., Borregaard, N. (2009) Ficolin-1 is present in a highly mobilizable subset of human neutrophil granules and associates with the cell surface after stimulation with fMLP. *J. Leukoc. Biol.* **86,** 1439–1449.
8. Honore, C., Rorvig, S., Hummelshoj, T., Skjoedt, M. O., Borregaard, N., Garred, P. (2010) Tethering of ficolin-1 to cell surfaces through recognition of sialic acid by the fibrinogen-like domain. *J. Leukoc. Biol.* **88,** 145–158.
9. Gout, E., Moriscot, C., Doni, A., Dumestre-Perard, C., Lacroix, M., Perard, J., Schoehn, G., Mantovani, A., Arlaud, G. J., Thielens, N. M. (2011) M-ficolin interacts with the long pentraxin PTX3: a novel case of crosstalk between soluble pattern-recognition molecules. *J. Immunol.* **186,** 5815–5822.
10. Kjaer, T. R., Hansen, A. G., Sorensen, U. B., Nielsen, O., Thiel, S., Jensenius, J. C. (2011) Investigations on the pattern recognition molecule M-ficolin: quantitative aspects of bacterial binding and leukocyte association. *J. Leukoc. Biol.* **90,** 425–437.

11. Thielens, N. M. (2011) The double life of M-ficolin: what functions when circulating in serum and tethered to leukocyte surfaces? *J. Leukoc. Biol.* **90,** 410–412.
12. Frankenberger, M., Schwaeble, W., Ziegler-Heitbrock, L. (2008) Expression of M-ficolin in human monocytes and macrophages. *Mol. Immunol.* **45,** 1424–1430.
13. Matsumoto, M., Atarashi, K., Umemoto, E., Furukawa, Y., Shigeta, A., Miyasaka, M., Hirata, T. (2005) CD43 functions as a ligand for E-selectin on activated T cells. *J. Immunol.* **175,** 8042–8050.
14. Fuhlbrigge, R. C., King, S. L., Sackstein, R., Kupper, T. S. (2006) CD43 is a ligand for E-selectin on CLA+ human T cells. *Blood* **107,** 1421–1426.
15. Carlow, D. A., Ziltener, H. J. (2006) CD43 deficiency has no impact in competitive in vivo assays of neutrophil or activated T cell recruitment efficiency. *J. Immunol.* **177,** 6450–6459.
16. Yago, T., Fu, J., McDaniel, J. M., Miner, J. J., McEver, R. P., Xia, L. (2010) Core 1-derived O-glycans are essential E-selectin ligands on neutrophils. *Proc. Natl. Acad. Sci. USA* **107,** 9204–9209.
17. Nusbaum, P., Laine, C., Bouaouina, M., Seveau, S., Cramer, E. M., Masse, J. M., Lesavre, P., Halbwachs-Mecarelli, L. (2005) Distinct signaling pathways are involved in leukosialin (CD43) down-regulation, membrane blebbing, and phospholipid scrambling during neutrophil apoptosis. *J. Biol. Chem.* **280,** 5843–5853.
18. Camous, L., Roumenina, L., Bigot, S., Brachemi, S., Fremeaux-Bacchi, V., Lesavre, P., Halbwachs-Mecarelli, L. (2011) Complement alternative pathway acts as a positive feedback amplification of neutrophil activation. *Blood* **117,** 1340–1349.
19. Seveau, S., Lopez, S., Lesavre, P., Guichard, J., Cramer, E. M., Halbwachs-Mecarelli, L. (1997) Leukosialin (CD43, sialophorin) redistribution in uropods of polarized neutrophils is induced by CD43 cross-linking by antibodies, by colchicine or by chemotactic peptides. *J. Cell Sci.* **110,** 1465–1475.
20. Hoffstein, S. T. (1979) Ultrastructural demonstration of calcium loss from local regions of the plasma membrane of surface-stimulated human granulocytes. *J. Immunol.* **123,** 1395–1402.
21. Naccache, P. H., Showell, H. J., Becker, E. L., Sha'afi, R. I. (1979) Involvement of membrane calcium in the response of rabbit neutrophils to chemotactic factors as evidenced by the fluorescence of chlorotetracycline. *J. Cell Biol.* **83,** 179–186.
22. Korchak, H. M., Vienne, K., Rutherford, L. E., Wilkenfeld, C., Finkelstein, M. C., Weissmann, G. (1984) Stimulus response coupling in the human neutrophil. II. Temporal analysis of changes in cytosolic calcium and calcium efflux. *J. Biol. Chem.* **259,** 4076–4082.
23. Naccache, P. H., Showell, H. J., Becker, E. L., Sha'afi, R. I. (1977) Transport of sodium, potassium, and calcium across rabbit polymorphonuclear leukocyte membranes. Effect of chemotactic factor. *J. Cell Biol.* **73,** 428–444.
24. Nathan, C., Xie, Q. W., Halbwachs-Mecarelli, L., Jin, W. W. (1993) Albumin inhibits neutrophil spreading and hydrogen peroxide release by blocking the shedding of CD43 (sialophorin, leukosialin). *J. Cell Biol.* **122,** 243–256.
25. Fukuda, M., Tsuboi, S. (1999) Mucin-type O-glycans and leukosialin. *Biochim. Biophys. Acta* **1455,** 205–217.
26. Piller, F., Piller, V., Fox, R. I., Fukuda, M. (1988) Human T-lymphocyte activation is associated with changes in O-glycan biosynthesis. *J. Biol. Chem.* **263,** 15146–15150.
27. Seveau, S., Keller, H., Maxfield, F. R., Piller, F., Halbwachs-Mecarelli, L. (2000) Neutrophil polarity and locomotion are associated with surface redistribution of leukosialin (CD43), an antiadhesive membrane molecule. *Blood* **95,** 2462–2470.
28. Mambole, A., Baruch, D., Nusbaum, P., Bigot, S., Suzuki, M., Lesavre, P., Fukuda, M., Halbwachs-Mecarelli, L. (2008) The cleavage of neutrophil leukosialin (CD43) by cathepsin G releases its extracellular domain and triggers its intramembrane proteolysis by presenilin/γ-secretase. *J. Biol. Chem.* **283,** 23627–23635.
29. Kuijpers, T. W., Hoogerwerf, M., Kuijpers, K. C., Schwartz, B. R., Harlan, J. M. (1992) Cross-linking of sialophorin (CD43) induces neutrophil aggregation in a CD18-dependent and a CD18-independent way. *J. Immunol.* **149,** 998–1003.
30. Rosenkranz, A. R., Majdic, O., Stockl, J., Pickl, W., Stockinger, H., Knapp, W. (1993) Induction of neutrophil homotypic adhesion via sialophorin (CD43), a surface sialoglycoprotein restricted to haemopoietic cells. *Immunology* **80,** 431–438.
31. Ostberg, J. R., Barth, R. K., Frelinger, J. G. (1998) The Roman God Janus: a paradigm for the function of CD43. *Immunol. Today* **19,** 546–550.
32. Woodman, R. C., Johnston, B., Hickey, M. J., Teoh, D., Reinhardt, P., Poon, B. Y., Kubes, P. (1998) The functional paradox of CD43 in leukocyte recruitment: a study using CD43-deficient mice. *J. Exp. Med.* **188,** 2181–2186.