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The Mammalian Actin-Binding Protein 1 Is Critical for Spreading and Intraluminal Crawling of Neutrophils under Flow Conditions

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Recently, the mammalian actin-binding protein 1 (mAbp1; Hip-55, SH3P7, debrin-like protein) was identified as a novel component of the β2 integrin-mediated signaling cascade during complement-mediated phagocytosis and firm adhesion of polymorphonuclear neutrophils (PMN) under physiological shear stress conditions. In this study, we found that the genetic ablation of mAbp1 severely compromised not only the induction of adhesion, but also subsequent spreading of leukocytes to the endothelium as assessed by intravital microscopy of inflamed vessels of the cremaster muscle of mice. In vitro studies using murine PMN confirmed that mAbp1 was required for β2 integrin-mediated spreading under shear stress conditions, whereas mAbp1 was dispensable for spreading under static conditions. Upon β2 integrin-mediated adhesion and chemotactic migration of human neutrophil-like differentiated HL-60 cells, mAbp1 was enriched at the leading edge of the polarized cell. Total internal reflection fluorescence microscopy revealed that mAbp1 formed propagating waves toward the front of the lamellipodium, which are characteristic for dynamic reorganization of the cytoskeleton. Accordingly, binding of mAbp1 to actin was increased upon β2 integrin-mediated adhesion, as shown by coimmunoprecipitation experiments. However, chemotactic migration under static conditions was unaffected in the absence of mAbp1. In contrast, the downregulation of mAbp1 by RNA interference technique in neutrophil-like differentiated HL-60 cells or the genetic ablation of mAbp1 in leukocytes led to defective migration under flow conditions in vitro and in inflamed cremaster muscle venules in the situation in vivo. In conclusion, mAbp1 is of fundamental importance for spreading and migration under shear stress conditions, which are critical prerequisites for efficient PMN extravasation during inflammation. The Journal of Immunology, 2012, 188: 4590–4601.

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croutment of polymorphonuclear neutrophils (PMN) to the site of inflammation follows a well-defined multistep adhesion cascade. It includes leukocyte capturing and rolling along the activated endothelium, firm adhesion, adhesion strengthening, spreading, and intraluminal crawling to the emigration sites where PMN eventually extravasate. Whereas selectins are the key molecules that allow PMN capture and rolling, adhesion is mainly dependent on adhesion molecules of the β2 integrin family (CD11/CD18) (1, 2). During rolling of PMN, chemokines presented by the endothelium as well as selectin-ligand interactions can activate the β2 integrins (inside-out signaling) by a shift of the conformation of the β2 integrin (3, 4). The β2 integrins exist in three different conformations, the low-affinity conformation that is mainly expressed on nonactivated PMN, as well as the intermediate-affinity and the high-affinity state that can be primarily found upon cellular activation (5).

Activation of β2 integrins is indispensable for different steps of the recruitment cascade. Whereas β2 integrins in the intermediate-affinity state contribute to slow rolling of PMN (4), firm adhesion under flow conditions requires the high-affinity conformation of the β2 integrins enabling PMN to remain adherent and withstand shear forces (1, 6). In addition, there is increasing evidence for different molecular functions of the β2 integrins LFA-1 (CD11a/CD18, αLβ2) and Mac-1 (CD11b/CD18, αMβ2) in the recruitment process. In vivo studies by Phillipson et al. (7–9) on adhesion and crawling of PMN in venules of the inflamed cremaster muscle in mice revealed that initial LFA-1–mediated adhesion was followed by crawling via Mac-1. In this study, crawling of the PMN to endothelial junctions was almost exclusively dependent on interaction between Mac-1 and endothelial ICAM-1 that eventually allowed efficient emigration out of the blood vessel.

Ligand binding of β2 integrins leads to integrin clustering and subsequent outside-in signaling, which contributes to adhesion strengthening and cell motility by controlling cytoskeletal rearrangements and cell polarization (10–13). Similar to classical immunoreceptor signaling, for example, via the TCR or the BCR, β2 integrin-mediated outside-in signaling causes phosphorylation

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Abbreviations used in this article: Arp2/3, actin-related protein 2 and 3; dHL-60, neutrophil-like differentiated HL-60; EGFP, enhanced GFP; Hem-1, hematopoietic protein 1; mAbp1, mammalian actin-binding protein 1; N-WASP, neural WASP; PLL, poly(L-lysine); PMN, polymorphonuclear neutrophil; RNAi, RNA interference; shRNA, short hairpin RNA; TIRF, total internal reflection fluorescence; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP family Verprolin-homologous protein; WIP, WASP-interacting protein.

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of ITAMs in the adaptor proteins DAP12 and Fc receptor γ-chain by Src family kinases (14, 15). The phosphorylation of ITAMs allows binding and activation of the nonreceptor tyrosine kinase Syk (16, 17). Previous studies have demonstrated a crucial role of Syk for different β2 integrin-mediated PMN functions, including slow leukocyte rolling and firm adhesion during inflammation in vivo, as well as the respiratory burst, cell spreading, migration, and phagocytosis (4, 16, 18–24). Signaling downstream of Syk involves several molecules, including phospholipase C-γ, the guanine nucleotide exchange factor Vav, the Src homology 2 domain-containing leukocyte phosphoprotein of 76 kDa, the adaptor protein Cbl, and the regulatory subunit p85 of the PI3K class 1 (18, 22, 25–30). In addition, the mammalian actin-binding protein 1 (mAbp1; HIP-55, SH3P7, debrin-like protein) has been shown to become phosphorylated by Src and Syk family kinases in fibroblasts and in lymphocytes (31–33).

The 55-kDa intracellular adaptor protein mAbp1 binds to actin via its N-terminal–located evolutionary conserved actin-depolymerizing factor homology domain and the neighboring helical domain (32, 34–36). At its C terminus, mAbp1 contains a Src homology 3 domain, as well as a proline-rich domain, which mediate protein-protein interactions (37, 38). In lymphocytes, mAbp1 has been shown to be critical for TCR-mediated signaling and Ag internalization, processing, and presentation in B cells (39, 40). Recently, we were able to demonstrate that mAbp1 was indispensable for Mac-1–mediated phagocytosis in PMN (21). Moreover, the genetic absence of mAbp1 caused a severe defect of PMN adhesion in TNF-α–stimulated cremaster muscle venules and in a microflow chamber under physiologic shear stress conditions in vitro, whereas adhesion was not affected under static conditions (21). The analysis of the underlying molecular mechanism revealed that mAbp1 was required for stabilization of CD18 clusters in the high-affinity conformation under flow conditions. Thus, mAbp1 seems to be involved in the reinforcement of the high-affinity conformation of the β2 integrins under shear stress conditions, thereby allowing adhesion strengthening, which is critical to withstand shear forces during adhesion (21).

As the high-affinity conformation of β2 integrins is known to be critical not only for the reinforcement of adhesion, that is, adhesion strengthening, but also plays an important role for other steps of the recruitment cascade (3), we studied whether mAbp1 has a functional impact on the induction of firm adhesion, spreading, and intraluminal crawling. We found that enhanced GFP (EGFP)-tagged mAbp1 (mAbp1-EGFP) was translocated to the lamellipodium of polarized neutrophil-like differentiated HL-60 (dHL-60) cells during β2 integrin-mediated adhesion and migration. In this study, mAbp1-EGFP formed wavelike structures that are known to be characteristic for actin reorganization. Accordingly, p85 integrin binding specifically increased the interaction of mAbp1 with actin. However, chemotactic migration and spreading of murine PMN under static conditions were not affected by the genetic absence of mAbp1. This was in sharp contrast to physiological shear stress conditions in which the induction of firm adhesion, spreading, and intraluminal crawling of leukocytes was significantly impaired in mAbp1−/−/− mice in vivo compared with mAbp1+/−/− control mice. Mechanotactic crawling on immobilized ICAM-1 was found to depend on Mac-1, as the crawling ability of isolated mAbp1−/−/− PMN was significantly reduced on truncated ICAM-1, which lacked the binding site for Mac-1. In summary, our data provide evidence that mAbp1 was critical for the induction of adhesion, spreading, and intraluminal crawling of PMN under flow conditions.

Materials and Methods

Mice

Mice carrying the Dbltail1 allele (mAbp1+−) and wild-type control mice maintained on the BALB/c background were provided by J. Wienands (Göttingen, Germany) (41). LFA-1−/− mice and wild-type control mice were maintained on the C57BL/6 background. LFA-1−/− mice were provided by N. Hogg (London, U.K.) (42). Animal experiments were institutionally approved.

Reagents and Abs

BSA, diisopropylfluorophosphate, DMSO, DTT, fibrinogen from human or mouse plasma, IMLP, HEPES, Percoll, piceatannol, PMSF, p-nitrophenyl phosphate, protease inhibitor mixture, sodium chloride, sodium deoxycholate, sodium metavanadate, sodium orthovanadate, Triton X-100, trypsin inhibitor, and Tween 20 were obtained from Sigma-Aldrich (Deisenhofen, Germany). Sodium fluoride and Trit were delivered by AppliChem (Darmstadt, Germany). RPMI 1640 medium, FCS, HBSS, penicillin/streptomycin, poly(L-lysine) (PLL), and PBS were purchased from Biochrom (Berlin, Germany). GFP-Trap-A beads were delivered by Chromotek (Munich, Germany). Human and murine ICAM-1 and murine P-selectin were obtained from R&D Systems (Minneapolis, MN). Keratinocyte-derived chemokine (KC; CXC1) was purchased from PeproTech (Hamburg, Germany). Murine TNF-α was obtained from Cell Signaling Technology (Danvers, MA). The mouse anti-Syk mAb (IgG2a, clone 4D10, sc1240), the mouse anti-Wiskott–Aldrich syndrome protein (WASP) mAb (IgG2a, clone B-9, sc13139), the goat anti-actin Ab (I-9, sc1616), and the rabbit anti–WASP-interacting protein (WIP) Ab (H-224, sc25533) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The goat anti-mAbp1 Ab (ab2836) was obtained from Abcam (Cambridge, U.K.). The mouse anti-mAbp1 mAb (IgG1, 612614) was purchased from BD Biosciences (Heidelberg, Germany). The HRP-conjugated goat anti-mouse IgG L chain Ab was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). The function-blocking mouse anti-CD18 mAb (Clone 3G8, sc7961), the goat anti-actin Ab (I-19, sc-1616), and the rabbit anti–WASP-interacting protein (WIP) Ab (H-224, sc25533) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The goat anti-mAbp1 Ab (ab2836) was obtained from Abcam (Cambridge, U.K.). The mouse anti-mAbp1 mAb (IgG1, 612614) was purchased from BD Biosciences (Heidelberg, Germany). The HRP-conjugated goat anti-mouse IgG, Alexa 488-conjugated goat anti-mouse IgG, Alexa 546-conjugated donkey anti-goat IgG, Alexa 488-conjugated donkey anti-rabbit IgG Ab, and Alexa 555-conjugated phalloidin were obtained from Invitrogen (Karlsruhe, Germany).

Isolation of bone marrow PMN and cell culture

Murine PMN were isolated, as described previously (28). The human myeloid HL-60 cells (ACC 3) and the murine WEHI-3B cells (ACC 26) were obtained from the German Research Centre for Biological Material (Braunschweig, Germany). Differentiation of neutrophil-like HL-60 cells was induced in culture using RPMI 1640 medium supplemented with 1.3% DMSO for 6 d. For pharmacological inhibition of Syk, cells were treated with 30 μM piceatannol for 30 min at 37 °C or vehicle (DMSO) for control.

Stable transduction of HL-60 cells

The mAbp1-EGFP expression vector was a gift of M. Deckert (INSERM, Université de Nice-Sophia-Antipolis, Nice, France). For viral transduction, the coding region of mAbp1-EGFP was subcloned into the FuVal lentiviral vector (Fu-mAbp1-EGFP). To generate virus-containing supernatant, HEK 293T cells (American Type Culture Collection; CRL-11268) were plated on 6-well plate culture plates in RPMI 1640 medium (supplemented with 10% FCS and 25 mM HEPEs) and transduced with the transducing vector Fu-mAbp1-EGFP, the packaging vector pCMVΔR8.9, and the envelope vector pVSV-G (43) using Lipofectamine 2000 (Invitrogen, Karlsruhe, Germany), according to the manufacturer’s instructions. Subsequently, HL-60 cells were incubated overnight in RPMI 1640 medium supplemented with 3% virus-containing supernatant. After 2 wk, transduced cells were sorted for fluorescence intensity using a FACS/Aria system (BD Biosciences, Heidelberg, Germany) and subcloned. Fluorescence intensity of the mAbp1-EGFP expressing HL-60 cell clones was measured using a FACSscan flow cytometer (BD Biosciences). The expression of the 85-kDa mAbp1-EGFP fusion protein was detected by Western blot technique (data not shown).

For the generation of the cell line that stably expressed a short hairpin (sh) RNA to downregulate mAbp1 by the RNA interference (RNAi) technique, we cloned the specific DNA oligonucleotide (5′-TCG AAA GTC CCC GAC CGA CTG GCC TTT CAA GAG AAC CCC AGT CGG TCG GGG ACT TCT TTT-3′; Biomers.net, Ulm, Germany) or a scrambled DNA oligonucleotide as control into the shRNA vector pSuppressorRetro
mAbp1 IS CRITICAL FOR INTRALUMINAL CRAWLING

To generate dHL-60 cells transiently expressing mAbp1-EGFP, a 400-μl aliquot of dHL-60 cells (2 × 10⁷ cells/ml) in RPMI 1640 medium was transfected to a Gene Pulser cuvette with an 0.4-cm electrode (BioRad, Hercules, CA) and mixed with 30 μg endotoxin-free mAbp1-EGFP expression vector. Samples were subjected to an electroporation pulse of 290 V and 1050 μF (EasyJet T electroporator; Equibio, Kent, U.K.).

Expression of ICAM-1(D1-2)-Fc fusion protein

The coding region of domains D1-D2 of ICAM-1 derived from cDNA of murine spleen was cloned into the expression vector pcDNA3.1 IgG (44). HEK 293T cells (American Type Culture Collection; CRL-11268) were transfected with the expression vector, and the fusion protein ICAM-1(D1-2)-Fc containing the human FcγI fragment was purified from supernatant (compare Fig. 5C).

Scanning confocal microscopy and total internal reflection fluorescence microscopy

Scanning confocal microscopy of cells fixed with 3.7% formaldehyde was conducted with a Zeiss LSM 410/Axiovert 135 microscope using a Zeiss 63×/1.2 water or a Zeiss 63×/1.4 oil objective (Zeiss, Göttingen, Germany). The quantitative analysis of the subcellular distribution of mAbp1 was performed offline by two different investigators in a blinded manner using confocal images. For total internal reflection fluorescence (TIRF) microscopy, dHL-60 cells expressing mAbp1-EGFP (1–2 × 10⁶) were suspended in medium (HBSS; Biochrom, Berlin, Germany) supplemented with 20 mM HEPES (pH 7.2), 0.25% (w/v) BSA, 1 mM Ca²⁺, 1 mM Mg²⁺, and 0.1% glucose, plated onto fibrinogen (250 μg/ml)-coated coverslips (Saur, Reutlingen, Germany), and incubated for 5 min at 37°C. Subsequently, cells were stimulated with 100 nM fMLP and analyzed at 37°C using an Axiovert 200 (Zeiss) microscope with a TIRF 488/561-nm laser system (VisiRon Systems, Cool-Snap-HQ camera (Roper Scientific), and Meta-morph software (Molecular Devices).

Spreading and migration under flow conditions

To analyze spreading of isolated murine PMN, IBIDI μ-slides VI 0.4 (IBIDI GmbH, Martinsried, Germany) were coated overnight with murine fibrinogen (50 μg/ml) or murine ICAM-1 (12.5 μg/ml). After rinsing with PBS, the chambers were filled with 3 × 10⁵ murine PMN/sample. After incubation of PMN for 10 min at 37°C with JMLP (1 μM) in adhesion medium (HBSS; Biochrom, Berlin, Germany) supplemented with 20 mM HEPES (pH 7.2), 0.25% (w/v) BSA, 0.1% (v/v) glucose, 1.2 mM Ca²⁺, and 1.0 mM Mg²⁺, shear stress (1 dyne/cm²) was applied for 10 min using a high-precision syringe pump (model KDS-232; KD Scientific, Holliston, MA). Samples were fixed with 3.7% formaldehyde, and microscopy was performed using a Zeiss Axiovert 200M microscope with a Plan-Apochromat original magnification ×63/1.4 oil objective, an AxioCam HR digital camera, and the AxioVision 4 software (Zeiss). Spreading of PMN was assessed by measuring the cell area using Image J software provided by the National Institutes of Health.

Crawling of dHL-60 cells or murine PMN under flow conditions was analyzed in IBIDI μ-slides VI 0.4 (IBIDI GmbH) or flow chambers (0.2 × 2-mm cross-section; VitroCom, Mountain Lake, NJ) coated with murine ICAM-1 (12.5 μg/ml) or human ICAM-1 (12.5 μg/ml) alone or with a combination of murine P-selectin (20 μg/ml), murine ICAM-1 (12.5 μg/ml), and KC (15 μg/ml), as indicated. A total of 3 × 10⁵ cells/sample was allowed to adhere for 10 min at 37°C. Crawling was recorded under shear stress (1 dyne/cm²) for 10 min at 37°C using the system mentioned above. Crawling was analyzed offline using Image J software plugin Manual Tracking and the Chemotaxis and Migration tool.

Spreading and migration under static conditions

Isolated murine PMN were seeded on coverslips coated with murine fibrinogen (50 μg/ml) and were stimulated for 30 min at 37°C with 100 ng/ml TNF-α or 1 mM Mn²⁺. Microscopic images of cells fixed with 3.7% formaldehyde were recorded using a Zeiss Axiovert 200M microscope with a Plan-Apochromat original magnification ×63/1.4 oil objective, an AxioCam HR digital camera, and the AxioVision 4 software (Zeiss). Cell area of spread cells was measured offline using Image J software.

Chemotactic migration of isolated murine PMN in response to a gradient of 10 μM JMLP was analyzed on immobilized murine fibrinogen (50 μg/ml) or murine ICAM-1 (12.5 μg/ml) in Zigmound chambers (45) using a Zeiss Axiovert 200M microscope equipped with Plan-Apochromat original magnification ×100/0.25 or Plan-Apochromat original magnification ×200/0.75 objectives, an AxioCam HR digital camera, and the AxioVision 4 software (Zeiss), as described previously (28). Tracking of PMN migration was carried out, as described above.

For time-lapse video microscopy of migrating dHL-60 cells transiently expressing mAbp1-EGFP, cells were seeded onto fibrinogen-coated IBIDI μ-slides Chemotaxis (IBIDI GmbH). Chemotactic migration in response to a gradient of 10 nM JMLP was recorded using a Plan-Apochromat 20×/0.75 objective (Zeiss) on the microscope system mentioned above.

Intravital microscopy

Intravital microscopy and recording of the cremaster muscle were conducted, as reported previously, using an upright microscope (model 512815/20; Leitz, Wetzlar, Germany) with a saline immersion objective (SW 40/0.75 NA) (19). Microvascular parameters were measured using a digital image processing system (46). For assessment of induction of adhesion and spreading of leukocytes, the cremaster muscle was superfused with 1 μM JMLP in bicarbonate-buffered saline (19). The number of adherent leukocytes in inflamed cremaster muscle venules was assessed, as described (47). Leukocytes were considered to be adherent when they were attached at the same position for >30 s. Rolling leukocyte flux fraction was defined as the percentage of rolling leukocytes to all leukocytes passing the same vessel in 1 min (47). Spreading of adherent leukocytes against the vessel wall was measured as a decrease in perpendicular diameter (19). To study intraluminal leukocyte crawling, murine TNF-α (500 ng/ml per mouse) was injected intrascrotally 2–4 h prior to intravital microscopy. Leukocyte crawling was defined as displacement of the cell by at least one cell diameter within the observation period of 10 min, and was analyzed offline using Image J software plugin Manual Tracking and the Chemotaxis and Migration tool (48). Percentage of cells migrating in the direction of blood flow was defined as the number of leukocytes migrating within an angle of 60° in the direction of blood flow in the venule compared with the number of all migrating leukocytes in the given vessel segment.

Coimmunoprecipitation

For coimmunoprecipitation experiments, dHL-60 cells stably expressing mAbp1-EGFP (8 × 10⁵/sample) or dHL-60 control cells suspended in medium (0.9% NaCl, 20 mM HEPES, 0.1% glucose, 1.2 mM Ca²⁺, 1 mM Mg²⁺) were seeded onto dishes coated with fibrinogen (250 μg/ml), ICAM-1 (12.5 μg/ml), truncated ICAM-1 (D1-2) (12.5 μg/ml) or PLL (100 μg/ml) and incubated at 37°C for 10 min. To inhibit β₂ integrin-mediated adhesion, cells were treated with a function-blocking Ab against CD18 (clone I4B, 50 μg/ml) for 20 min at room temperature prior to the onset of the experiment. Adhesion of cells was induced by addition of 1 mM Mn²⁺. After 15 min, cells were lysed using a modified radio-immunoprecipitation assay buffer without SDS (25 mM Tris [pH 7.5], 150 mM NaCl, 1% [v/v] Triton X-100, 1% [w/v] sodium deoxycholate, 1 mM DTT, 1 mM diisopropyl fluorophosphate, 100 μM sodium orthovanadate, 100 mM NaF, 1 mM PMSE, 0.25 mM sodium metavanadate, protease inhibitor mixture [Sigma-Aldrich; 1:1000], 50 μg/ml trypsin inhibitor, 10 μM p-nitrophenyl phosphate) for 30 min at 4°C. GFP-Trap beads (Chromotek, Munich, Germany) were added and coimmunoprecipitation was performed, according to manufacturer’s protocol. Finally, beads were resuspended in SDS sample buffer and boiled for 10 min at 95°C. Western blot was performed using the mouse anti-mAbp1 mAb (BD Biosciences, Heidelberg, Germany) or the goat anti-actin Ab (sc-1616; Santa Cruz Biotechnology) and secondary HRP-conjugated goat anti-mouse IgG L chain (Jackson ImmunoResearch Laboratories) or rabbit anti-goat IgG (Calbiochem, Darmstadt, Germany) Abs, respectively.

Statistical analysis

Data shown represent means ± SD or means ± SEM, respectively. Statistical significance was determined using Student t test, the Mann–Whitney rank sum test, or Kruskal–Wallis ANOVA test, respectively. A p value < 0.05 was considered statistically significant.

Results

Localization of mAbp1 during leukocyte adhesion and migration

During β₂ integrin-mediated phagocytosis of complement-opsonized Escherichia coli, mAbp1 has been shown to be dynamically enriched at the phagocytic cup of dHL-60 cells and primary murine PMN. This localization of mAbp1 at the phago-
cytic cup depended on active Syk (21). In this work, we studied the subcellular localization of mAbp1 during β2 integrin-mediated adhesion and migration. By means of confocal microscopy, we found that upon induction of adhesion to immobilized fibrinogen by the bacteria-derived tripeptide fMLP (100 nM), EGFP-tagged mAbp1 (mAbp1-EGFP) was markedly enriched at the leading edge of polarized dHL-60 cells and colocalized with F-actin at the lamellipodium (Fig. 1A). The enrichment of mAbp1 at the leading edge was compromised when Syk was pharmacologically inhibited by piceatannol (Fig. 1B). The quantitative analysis revealed that the percentage of polarized cells with a localization of mAbp1 at the cell front was significantly reduced from 65.7% of control

**FIGURE 1.** Localization of mAbp1, WASP, and WIP at the leading edge was dependent on active Syk. (A) Confocal microscopy image of adherent and polarized dHL-60 cells transiently transfected with mAbp1-EGFP (mAbp1-EGFP) and stimulated with fMLP (100 nM). F-actin was stained with Alexa 555-conjugated phalloidin (red). mAbp1-EGFP (green) was enriched at the leading edge (arrows) and colocalized with F-actin (yellow, merged image). (B, D, and E) Confocal microscopy images of dHL-60 cells incubated with 30 μM piceatannol (PIC) or vehicle for control (control). Adhesion and polarization of dHL-60 cells on immobilized fibrinogen were induced by fMLP (100 nM) for 30 min. (B) Indirect fluorescence staining of Syk was performed using a mouse anti-Syk mAb and a secondary Alexa 488-conjugated anti-mouse IgG Ab (green). mAbp1 was assessed using a goat anti-mAbp1 Ab and a secondary Alexa 546-conjugated anti-goat IgG Ab (red). Upon stimulation, Syk and mAbp1 were enriched at the leading edge (arrows) of polarized dHL-60 cells. The merged images demonstrate colocalization (yellow). Piceatannol treatment induced the formation of multiple lamellipodia (arrowheads), and dHL-60 cells showed a homogenous distribution of Syk and mAbp1. Images are representative for three independent experiments. Scale bar, 10 μm. (C) Quantitative analysis of subcellular mAbp1 distribution. Polarized cells that formed at least one lamellipodium upon fMLP stimulation were counted according to the subcellular distribution of mAbp1. n = 239 (control); n = 275 (PIC) in three independent experiments. Means ± SD; homo = homogeneous; *p < 0.05. (D and E) Indirect fluorescence staining of WASP was performed using a mouse anti-WASP mAb and a secondary Alexa 488-conjugated anti-mouse IgG Ab (green). mAbp1 was assessed using a goat anti-mAbp1 Ab and a secondary Alexa 546-conjugated anti-goat IgG Ab (red). Upon stimulation, WASP and WIP were enriched at the leading edge (arrows) of polarized dHL-60 cells and colocalized with F-actin (yellow, merged images). Piceatannol treatment induced the formation of multiple lamellipodia (arrowheads), and proper localization of WASP and WIP at the lamellipodia was diminished. Images are representative for four independent experiments. Scale bar, 10 μm. (F) Box-whisker plot of Pearson’s correlation coefficient of F-actin and WASP or F-actin and WIP, respectively. Median numbers are indicated. n = 73 (WASP; control), n = 78 (WASP; PIC) of four independent experiments. *p < 0.05.
cells to 36.7% of cells treated with piceatannol (Fig. 1C), suggesting that active Syk was required for the proper localization of mAbp1. Similarly, the inhibition of Syk interfered with the proper localization of two additional cytoskeleton-associated proteins, namely the WASP and WIP at the lamellipodium of polarized dHL-60 cells (Fig. 1D, 1E). WASP is known to activate the actin-related protein 2 and 3 (Arp2/3) complex, which is indispensable for generation and reorganization of actin. Activity and stability of WASP are regulated by WIP (49–52). Quantitative analysis of the colocalization between WASP and WIP with F-actin by calculating the Pearson’s correlation coefficient revealed that inhibition of Syk resulted in a diminished colocalization of these proteins with F-actin (Fig. 1F). Thus, the three proteins, WASP, WIP, and mAbp1, seem to represent cytoskeleton-associated components of the β2 integrin-mediated signaling complex downstream of Syk.

To study the subcellular dynamics of mAbp1 during migration in detail, we performed time-lapse video microscopy of live dHL-60 cells expressing EGFP-tagged mAbp1 (mAbp1-EGFP). We found that mAbp1-EGFP was enriched at the leading edge of cells migrating toward a gradient of the chemoattractant fMLP (10 nM) on immobilized fibrinogen (Fig. 2A). For further analysis, we used a lentivirally transduced dHL-60 cell clone that stably expressed mAbp1-EGFP comparable to the endogenous mAbp1 level (21). Using TIRF microscopy of polarized mAbp1-EGFP expressing dHL-60 cells at the plasma membrane with high resolution. Using this approach, we identified wavelike structures formed by dynamic accumulation of mAbp1 toward the leading edge of the cell (Fig. 2B, Supplemental Video 1). Such propagating waves have been described previously for actin and other actin-associated proteins such as myosin-II, the Arp2/3 complex, and coronin in Dictyostelium, and for hematopoietic protein 1 (Hem-1) in dHL-60 cells (53–56). Therefore, we studied whether mAbp1 may link the β2 integrin function to the actin cytoskeleton. dHL-60 cells expressing mAbp1-EGFP were left unstimulated in suspension for control or were seeded on immobilized fibrinogen or ICAM-1. Adhesion was induced by addition of Mn2+ (1 mM), which is known to stabilize the high-affinity conformation of β2 integrins (57). Coimmunoprecipitation technique revealed that the mAbp1-actin interaction was markedly induced upon β2 integrin-mediated adhesion on fibrinogen or ICAM-1 compared with cells in suspension or cells exposed to PLL for control (Fig. 2C). An induction of the mAbp1-actin interaction was also observed in cells adherent to truncated ICAM-1 (ICAM-1 D1-2), which lacked the binding domains of the β2 integrin Mac-1 (44). In this study, adhesion was mediated by an interaction of the β2 integrin LFA-1 and ICAM-1 (D1-2). The mAbp1-actin interaction was markedly decreased when the cells were exposed to ICAM-1 in the presence of a function-
Role of mAbp1 for leukocyte adhesion and spreading in vitro and in vivo

Cytoskeletal rearrangements are indispensable for leukocyte recruitment, allowing firm adhesion, spreading, and migration on the endothelium under flow conditions (58). To elucidate the functional relevance of our findings for leukocyte recruitment, we studied leukocyte rolling, adhesion, and spreading in unstimulated and fMLP-stimulated cremaster muscle venules in mAbp1−/− and mAbp1+/+ mice using intravital microscopy. Microvascular parameters are presented in Table I and were comparable between mAbp1−/− and mAbp1+/+ mice in diameter of the venules, centerline velocity of the blood flow, and wall shear rate. Systemic leukocyte counts per microliter as assessed in whole blood samples were significantly higher in mAbp1+/+ mice compared with mAbp1+/+ mice. In unstimulated cremaster muscle venules, rolling flux fraction observed within the first 30 min after cremaster muscle exteriorization was similar between the two groups (Fig. 3A, 0.32 ± 0.02 in mAbp1+/+ mice and 0.33 ± 0.03 in mAbp1−/− mice), suggesting that mAbp1 was dispensable for leukocyte rolling at least in this setting, where rolling was induced by surgical trauma and dependent on P-selectin (48). Next, we analyzed leukocyte adhesion in unstimulated cremaster muscle venules (pre) and found no significant difference in the number of adherent leukocytes between mAbp1+/+ mice (200 ± 24/mm²) and mAbp1−/− mice (236 ± 28/mm²) (Fig. 3C, pre). Because leukocyte adhesion greatly depends on systemic leukocyte counts, we calculated the adhesion efficiency (adherent leukocytes per mm²/systemic leukocyte count per μl), which nevertheless was not affected in mAbp1−/− mice (0.032 ± 0.004) compared with mAbp1+/+ mice (0.039 ± 0.005) (Fig. 3B). However, upon local stimulation of the cremaster muscle by fMLP (1 μM) superfusion, the number of adherent leukocytes increased in mAbp1+/+ mice within 15 min (Fig. 3C, 476 ± 62/mm²) accompanied by a concomitant decrease in rolling flux fraction (Fig. 3D, −15.8 ± 2.1%). This response was almost completely absent in mAbp1−/− mice (251 ± 44 adherent leukocytes/mm² and −7.1 ± 2.3% rolling flux fraction after 15 min), suggesting that fMLP-induced leukocyte adhesion was dependent on mAbp1. Next, we studied leukocyte spreading measured as cell flattening against the vessel wall in vivo, that is, the decrease of the perpendicular leukocyte diameter (Fig. 4A). During fMLP stimulation, the cell diameter of adherent leukocytes in mAbp1+/+ mice continuously decreased from 6.44 ± 0.13 μm to 5.15 ± 0.11 μm after 15 min, representing cell spreading, as expected. In contrast, there was no spreading detectable in response to fMLP in mAbp1−/− mice from 6.41 ± 0.10 μm to 6.53 ± 0.13 μm after 15 min. These findings clearly indicated a crucial role for mAbp1 in PMN recruitment, namely induction of adhesion and spreading in vivo.

To further characterize the impact of mAbp1 on spreading, we performed in vitro flow chamber assays using bone marrow-derived PMN from mAbp1−/− mice and mAbp1+/+ mice (Fig. 4B). In this setting, an increase of the cell area indicates cell spreading on the substrate. Compared with mAbp1+/+ PMN, the cell area of mAbp1−/− PMN adherent to immobilized fibrinogen or ICAM-1 was significantly lower, confirming the spreading defect under physiological shear stress conditions (1 dyne/cm²) in response to

Table I. Hemodynamic parameters in fMLP-stimulated cremaster muscle venules

<table>
<thead>
<tr>
<th></th>
<th>Mice (n)</th>
<th>Venules (n)</th>
<th>Diameter (μm)</th>
<th>Centerline Velocity (μm/s)</th>
<th>Wall Shear Rate (s⁻¹)</th>
<th>Systemic Leukocyte Counts (μl⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>mAbp1+/+</td>
<td>7</td>
<td>26</td>
<td>29 ± 1</td>
<td>1750 ± 120</td>
<td>1500 ± 90</td>
<td>5100 ± 300</td>
</tr>
<tr>
<td>mAbp1−/−</td>
<td>6</td>
<td>19</td>
<td>31 ± 1</td>
<td>1700 ± 120</td>
<td>1400 ± 100</td>
<td>7300 ± 200</td>
</tr>
</tbody>
</table>

All values are means ± SEM.

FIGURE 3. Leukocyte rolling and adhesion in unstimulated and fMLP-stimulated cremaster muscle venules in vivo. Initial rolling flux fraction (A) and adhesion efficiency (B) of leukocytes before local superfusion of cremaster muscle. Adhesion (C) and rolling flux fraction (D) of leukocytes before (pre) and during local stimulation of cremaster muscle venules of mAbp1−/− and mAbp1+/+ mice with fMLP (1 μM). Initial rolling flux fraction (A) and adhesion efficiency (B) of leukocytes were not altered in the genetic absence of mAbp1 compared with mAbp1+/+ mice. (C) Induction of leukocyte adhesion upon stimulation with fMLP was impaired in mAbp1−/− mice, resulting in a (D) decreased change of rolling flux fraction. (A–D) n = 7 mAbp1+/+ mice, n = 6 mAbp1−/− mice, means ± SEM, *p < 0.05.
mAbp1 IS CRITICAL FOR INTRALUMINAL CRAWLING

**FIGURE 4.** Leukocyte spreading was impaired in absence of mAbp1 under flow conditions. (A) Diameters of adherent leukocytes from mAbp1−/− and mAbp1+/+ mice were measured perpendicular to the vessel at indicated time points during superfusion with fMLP (1 μM). Adherent leukocytes from mAbp1−/− mice failed to spread against the vessel wall, resulting in a constant cell diameter, whereas this percentage was significantly reduced from 53.4 ± 22.8% in mAbp1+/+ PMN to 31.5 ± 12.9% in mAbp1−/− PMN (Fig. 4C, 4D). Thus, mAbp1 was only indispensable for spreading under shear stress conditions, but not required under static conditions.

**Impact of mAbp1 for leukocyte migration and crawling under shear stress conditions**

Next, we studied crawling of isolated murine PMN from mAbp1−/− mice and mAbp1+/+ mice using an in vitro flow chamber assay. Analysis of mechanotactic migration of murine PMN on immobilized ICAM-1 under physiological shear stress conditions (1 dyne/cm²) revealed that mAbp1+/+ PMN migrated under flow conditions. In contrast, the majority of mAbp1−/− PMN showed some motility, but no significant net movement (Fig. 5A, Supplemental Video 2). Quantitative analysis showed that the majority of mAbp1+/+ PMN performed substantial migration under flow conditions, whereas this percentage was significantly reduced from 53.4 ± 22.8% in mAbp1+/+ PMN to 31.5 ± 12.9% in mAbp1−/− PMN (Fig. 5B, left panel). As shown in Fig. 5B (right panel), the ability of adherent mAbp1+/+ PMN to migrate under shear stress conditions was severely compromised on truncated ICAM-1 (ICAM-1 D1-2, Fig. 5C) lacking the binding domains of the β2 integrin Mac-1 (44). In this setting, cellular interactions were only possible via binding of LFA-1 to ICAM-1. Subsequently, the percentage of migrating mAbp1+/+ PMN was significantly reduced to similar levels, as seen for mAbp1−/− PMN on immobilized full-length ICAM-1, indicating that mechanotactic crawling was dependent on Mac-1, as expected. To confirm this finding, we studied mechanotactic crawling on full-length ICAM-1 using LFA-1−/− PMN. The percentage of migrating LFA-1−/− PMN was similar compared with LFA-1+/+ PMN (Fig. 5D), indicating that crawling of PMN under shear stress conditions was independent of LFA-1.

Detailed analysis of mechanotactic migration of isolated murine PMN on immobilized ICAM-1 and KC revealed that not only the percentage of migrating cells was diminished. Also, the migration velocity of the remaining mAbp1−/− PMN that crawled during 10 min of physiological shear stress of 1 dyne/cm² was significantly reduced from 1.9 ± 0.8 μm/min in mAbp1+/+ PMN to 1.4 ± 0.8 μm/min in mAbp1−/− PMN (Fig. 6A). Moreover, calculation of the frequency distribution of the migration velocity revealed that there were two groups of crawling mAbp1+/+ PMN. One group crawled at a low migration velocity (<2 μm/min), and the second group migrated at a higher migration velocity of >2 μm/min. In contrast, the majority of mAbp1−/− PMN crawled at the low migration velocity of <2 μm/min.

To study intraluminal crawling of leukocytes in vivo, we performed intravital microscopy of TNF-α-stimulated cremaster muscle venules of mAbp1−/− mice and mAbp1+/+ mice as control. The analysis of the migration tracks of leukocytes that crawled on the inflamed endothelium showed that wild-type leukocytes of mAbp1+/+ mice preferentially migrated perpendicular or against the direction of blood flow, as described by Phillipson et al. (59), for mechanotactic crawling of murine PMN under flow conditions in vitro and in vivo (Fig. 6B, Supplemental Video 3; WT). In contrast, a high number of mAbp1−/− leukocytes crawled in the direction of blood flow (Fig. 6B, Supplemental Video 3; KO). Quantitative analysis revealed that the percentage of cells that were not able to crawl perpendicular or against the direction of blood flow, but crawled in the direction of blood flow, was significantly increased in mAbp1−/− mice to 46.3 ± 6.2% compared with 26.0 ± 6.4% in mAbp1+/+ mice (Fig. 6C).
To analyze the impact of mAbp1 in the human system, we studied crawling of dHL-60 cells under shear stress conditions at 1 dyne/cm² on immobilized ICAM-1 (Fig. 6D). In accordance with the results obtained from murine PMN, dHL-60 cells downregulated mAbp1 expression by RNAi technique (shAbp1) to 19% compared with control cells expressing a scrambled RNA (Fig. 6E, 6F). This was also true for the calculated Euclidean distance. Further analysis of the single migration tracks and frequency distribution of migration angles (rose diagram) showed that the downregulation of mAbp1 in dHL-60 cells prevented the ability of the cells to migrate against or perpendicular to the direction of flow almost completely, whereas the majority of the control cells again crawled perpendicular or against the direction of flow (Fig. 6D), confirming the in vivo findings in the human system. These results indicated a crucial role of mAbp1 in mechanotactic crawling of PMN under shear stress conditions in vitro and in the situation in vivo.

To examine the role of mAbp1 in chemotactic migration, we used Zigmond chambers with fMLP (10 μM) as chemoattractant. We found that the genetic absence of mAbp1 in PMN did not affect chemotactic migration toward the gradient of fMLP on immobilized fibrinogen when compared with mAbp1+/+ control PMN (Fig. 7A). This was also true for migration velocity and mean accumulated and Euclidean distance (Fig. 7C, upper panel), suggesting that chemotactic migration under static in vitro conditions did not require mAbp1. Similar results were obtained on immobilized ICAM-1. In this study, chemotactic migration and migratory parameters of PMN were again not affected in the absence of mAbp1 (Fig. 7B, 7C, lower panel). Taken together, our results demonstrate that mAbp1 was indispensable for spreading and Mac-1–dependent intraluminal crawling under shear stress conditions, but not required for spreading or chemotactic migration under static conditions.

Discussion

The present study indicates a role of mAbp1 for induction of β₂ integrin-mediated adhesion, spreading, and intraluminal crawling of PMN under flow conditions. In dHL-60 cells, mAbp1 was translocated to the leading edge during Mac-1–mediated adhesion and migration on immobilized fibrinogen. This enrichment was dependent on active Syk, indicating that mAbp1 is part of the Syk-controlled signaling complex, which stabilizes the leading edge of migrating PMN (22, 28, 60). Also, WASP and WIP showed compromised enrichment at the leading edge and impaired colocalization with actin upon inhibition of Syk. Thus, mAbp1, WASP, and WIP may represent cytoskeleton-associated components of the signaling cascade downstream of Syk, which is known to become activated upon ligand binding of β₂ integrins controlling postadhesion events in PMN (14–16, 18, 21). Similar to WASP and WIP, mAbp1 colocalized with F-actin at the lamellipodium. This finding is in accordance with a previous study showing polarized recruitment of mAbp1 to the cell periphery of migrating fibroblasts, where it colocalized with F-actin. In this study, the accumulation of mAbp1 occurred predominantly at sites of high F-actin dynamics at the leading edge (35).

By means of TIRF microscopy, we identified an even more complex pattern of mAbp1 distribution forming propagating waves of mAbp1-EGFP toward the lamellipodium of polarized cells. These waves of mAbp1 resembled the discrete nonuniform propagating waves of Hem-1 in fMLP-simulated HL-60 cells found by Weiner et al. (56, 61). Hem-1 is a hematopoietic-specific component of the Arp2/3 complex-activating WASP family Verprolin-homologous protein (WAVE) complex. It was found to accumulate at the leading edge of fMLP-treated HL-60 cells, and TIRF microscopy revealed that Hem-1 forms sharp actin-based wavefronts at the plasma membrane of the lamellipodium, probably resulting from rapid Hem-1 recruitment and release (56, 61). The Arp2/3 complex, which catalyzes nucleation and branching of actin filaments, is controlled by nucleation-promoting factors like WASP and WAVE family proteins (52). In contrast to its yeast homolog...
Abp1p, mammalian Abp1 exerts no nucleation-promoting function, and mAbp1 cannot activate the Arp2/3 complex directly. However, Pinyol et al. (34, 52, 62) showed that mAbp1 directly interacted with the Arp2/3 complex activator neural WASP (N-WASP) in neuronal cells and thereby promoted actin polymerization together with N-WASP. Furthermore, mAbp1 was shown to cooperate with the small GTPase Cdc42, which in turn is known to mediate WASP/N-WASP activation. Because mAbp1 is part of the N-WASP/Arp2/3 complex, waves of mAbp1 might reflect dynamics of the WASP/N-WASP complex at the lamellipodium similar to Hem-1 in the WAVE complex.

Functional analysis revealed a substantial impact of mAbp1 for the induction of leukocyte adhesion to the endothelium in fMLP-stimulated cremaster muscle venules and for subsequent spreading against the vessel wall, as both responses were almost completely absent in mAbp1−/− mice. In contrast, the genetic absence of mAbp1 did not interfere with P-selectin–mediated rolling, as the initial rolling flux fraction was not different between mAbp1+/+ and mAbp1−/− mice.
and mAbp1<sup>−/−</sup> mice, providing evidence that the initial steps of leukocyte recruitment were not disturbed by the genetic absence of mAbp1. The observed defect in leukocyte spreading in the inflamed cremaster of mAbp1<sup>−/−</sup> mice resembles the spreading defect of Syk<sup>−/−</sup> leukocytes in vivo, as reported earlier (19). Interestingly, Syk was found to be indispensable for adhesion and spreading even under static in vitro conditions, whereas mAbp1 was not required for adhesion or spreading under these conditions (19, 21). Thus, unlike Syk, mAbp1 seems to be only required when mechanical force is applied on the β<sub>2</sub> integrins.

Intraluminal crawling of neutrophils is a postarrest step of the recruitment cascade, which has been shown to be mediated by interaction of Mac-1 and endothelial ICAM-1 in mice in vivo (8, 48, 59). This was also true in our experimental setting using isolated murine PMN migrating on immobilized ICAM-1 under shear stress conditions. In this study, the percentage of migrating cells was severely compromised in mAbp1<sup>−/−</sup> PMN, suggesting an impact of mAbp1 for Mac-1–mediated postarrest steps in neutrophil recruitment. Further analysis revealed a similar defect in the human system when mAbp1 was downregulated by RNAi technique. In addition to the overall migration capacity, the genetic ablation of mAbp1 in mice or the downregulation of mAbp1 by RNAi technique in dHL-60 cells interfered with the ability of the cells to migrate against and perpendicular to the direction of flow. In contrast, this behavior was typical for mAbp1<sup>+/+</sup> leukocytes in the inflamed cremaster muscle model in vivo as well as in control dHL-60 cells in an in vitro flow chamber assay. Phillipson et al. (8, 59) described perpendicular intraluminal crawling of PMN as a mechanism to find emigration sites. In Vav<sup>−/−</sup> mice, PMN were not able to migrate perpendicular to the direction of blood flow, but crawled with the flow. Our findings on the impact of mAbp1 on migration under shear stress resembled the observation on Vav<sup>−/−</sup> PMN. Similar to mAbp1, Vav is known to be important for neutrophil migration and was found to be part of the signaling complex downstream of Syk (28), arguing for an important impact of β<sub>2</sub> integrin-mediated signaling via Syk for Mac-1–dependent intraluminal crawling.

Analysis of spreading and chemotactic migration of mAbp1<sup>−/−</sup> PMN under static conditions revealed no differences compared with control cells. Similarly, the downregulation of the mAbp1 ortholog Dabp1 in Dictyostelium discoideum by RNAi technique (siDabp1) did not affect cell migration (63). In contrast, the overexpression of Dabp1 led to an impaired migration, suggesting a functional compensation by other signaling molecules in the siDabp1 cells (63). A compensatory mechanism could also work in mAbp1<sup>−/−</sup> PMN, allowing adhesion, spreading, and migration at least under static conditions. In yeast, Abp1p has redundant functions with the protein Sla2p (34). Less is known about the mammalian ortholog of Sla2p, the Huntingtin-interacting protein-1–related protein (Hip1R). However, it has been shown to bind F-actin, to associate

![FIGURE 7](http://www.jimmunol.org/Download/FIGURE_7.png)

**FIGURE 7.** mAbp1 was dispensable for chemotactic migration of PMN under static conditions. (A–C) Trajectories and migratory parameters of murine PMN isolated from mAbp1<sup>+/+</sup> and mAbp1<sup>−/−</sup> mice migrating in response to a gradient of 10 μM fMLP (◆) on immobilized fibrinogen (A, upper panel) or murine ICAM-1 (B, lower panel), respectively. The final positions relative to their original starting point are indicated by a filled circle (A, left panels). In the rose diagram (A, right panels), the area of each sector is proportional to the frequency of the migration vectors of the tracked cells pointed in the respective direction. Under static conditions, chemotactic migration on immobilized fibrinogen or ICAM-1 was similar between the two groups. n = 4 on immobilized fibrinogen, n = 3 on immobilized ICAM-1. (C) Means ± SD.
with clathrin-coated vesicles, and to form a complex with cortactin, which acts as a negative regulator of actin polymerization in HeLa cells, suggesting that Hip1R may compensate for the absence of mAbp1 during chemotactic migration (64).

Growing evidence supports the concept that mechanical force may be required for the development of adhesion sites by inducing recruitment of additional proteins. Force can be generated either internally, for example, by actin polymerization, or externally, for example, by shear stress (65). In this study, we demonstrated that actin binding of mAbp1 was enhanced upon induction of adhesion via β2 integrins. It may be reasonable to speculate that mAbp1 might be a novel component of the adhesion protein network that is necessary to connect the ligand-bound β2 integrins to the cytoskeleton, which in turn is indispensable to resist shear forces during adhesion, spreading, and migration under flow conditions. Accordingly, our data implicate that mAbp1 exerts its function rather under the influence of high external forces like exposure to shear stress than upon generation of moderate internal forces such as shear stress.

Taken together, we found that mAbp1 was critical for β2 integrin-mediated poststress functions of PMN, namely spreading and crawling under flow conditions. However, mAbp1 was only dispensable when external forces were applied to the integrin, whereas spreading and migration were normal under static conditions. Accordingly, mAbp1 seems to be critical for the transmission of mechanical force from the ligand-bound β2 integrin to the actin cytoskeleton, which is not only crucial for induction of adhesion under flow conditions, but also for intraluminal spreading and crawling of neutrophils during their recruitment to sites of inflammation.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Video Legends

Video 1. Subcellular dynamics of mAbp1. Live-cell imaging using TIRF microscopy of an adherent and polarized dHL-60 cell stably expressing mAbp1-EGFP (mAbp1-EGFP). Images were recorded every second and mAbp1-EGFP fluorescence intensity was displayed in pseudocolors for better visualization using the Green Fire Blue LUT from ImageJ software. mAbp1-EGFP was translocated to the lamellipodium in propagating waves. Bar = 10 μm.

Video 2. mAbp1 is critical for mechanotactic crawling under flow conditions. Time-lapse video microscopy of migrating murine mAbp1^{+/+} (WT) and mAbp1^{-/-} (KO) PMN in a flow chamber coated with murine ICAM-1 (12.5 μg/ml) upon stimulation with fMLP (1 μM) at physiological shear stress (1 dyne/cm², direction of flow: right to left). The used frame-rate was 5 seconds. Single cell tracks were generated using the ImageJ plugin Manual Tracking. The genetic absence of mAbp1 (KO) diminished the ability of PMN to migrate under flow conditions compared to mAbp1^{+/+} PMN (WT). Bar = 10 μm.

Video 3. Direction of intraluminal crawling of leukocytes was affected by the genetic absence of mAbp1. Intravital microscopy of inflamed venules of TNFα-treated cremaster muscle from mAbp1^{+/+} (WT) and mAbp1^{-/-} (KO) mice. The used frame-rate was 3 seconds. Single cell tracks from leukocytes that crawled on the endothelium were generated using the ImageJ plugin Manual Tracking. The genetic absence of mAbp1 (KO) compromised the ability of leukocytes to migrate perpendicular or against the direction of blood flow compared to leukocytes from mAbp1^{+/+} mice (WT). Bar = 50 μm.