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RESEARCH

# MOSPD2 regulates the activation state of αLβ2 integrin to control monocyte migration: applicability for treatment of chronic inflammatory diseases

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## Abstract

Monocytes are innate immune cells that drive the chronicity of various inflammatory diseases. Monocyte migration to inflamed tissues involves multiple steps of interaction with the vascular endothelium and the extracellular matrix (ECM), a process mediated through conformational transitions in cell surface integrins. We previously described motile sperm domain-containing protein 2 (MOSPD2) as a surface protein expressed on myeloid cells that is essential for the migration of monocytes and a key regulator of inflammation. Investigating MOSPD2's mechanism of action, we assessed whether it plays a role in regulating integrin activation and monocyte adhesion. Data show that silencing of MOSPD2 expression in the THP-1 monocytic cell line significantly increased cell adhesion to various ECM molecules. Employing IW-601, a humanized anti-human MOSDP2 monoclonal antibody, on primary human monocytes increased adhesion to ECM molecules as well as to adhesion molecules. At the molecular level, silencing of MOSPD2 or blocking MOSPD2 using IW-601 led to a transition in integrin αLβ2 (CD11a/CD18, LFA-1) conformation into an active high-affinity binding form and to the induction of adhesion-associated signaling pathways. Co-immunoprecipitation experiments showed that MOSPD2 binds integrin-β2 (CD18), but not integrin-β1 (CD29). Our results reveal a novel mechanism controlling monocyte migration, in which MOSPD2 acts as an adhesion checkpoint that governs the balance between monocyte adhesion and release. By demonstrating the inhibitory effect of IW-601 on the migration of primary monocytes isolated from patients with chronic inflammatory diseases, we provide proof of concept for translating MOSPD2's mechanism into a potential treatment for inflammatory diseases, further supported by in vivo data in models of RA and IBD.

**Keywords** Adhesion · Migration · αLβ2 · MOSPD2 · Inflammation · Checkpoint

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## Introduction

Leukocyte migration from the periphery to sites of inflammation is a multistage process in which integrins mediate both cell adhesion to endothelial cells and travel through the extracellular matrix (ECM). Integrins on leukocytes can bind a variety of ligands, including intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs), which are upregulated on activated endothelial cells, as well as components of the ECM such as fibronectin, collagen, and laminin. Integrins are heterodimers comprised of α/β-subunits, which are expressed on the transmembrane surface. Out of eight different types of β-subunits, β1 (CD29), β2 (CD18), β3, and β7 can be found on leukocytes, whereas the β2 and β7 subfamilies are expressed exclusively by leukocytes [1–3].

Structural and signaling studies have shown that integrins can adopt three conformational states: bent-closed, extended-closed, and extended-open: inactive integrins display a bent-closed conformation, which precludes engagement with counterpart ligands. Upon ligation of chemokine receptors or TLRs on leukocytes, an inside-out signaling recruits the intracellular adaptors, kindlin-3 and talin, to the β-subunit of the integrin and induces a transition to the intermediate extended-closed conformation and then to the high-affinity extended-open conformation. Driven by the binding of the high-affinity form of the integrin to extracellular ligands, the induction of outside-in signaling promotes F-actin cross-linkage to establish the "integrin adhesome" characterized by integrin clustering and strengthened integrin-ligand adhesion [4].

Motile sperm-domain containing protein 2 (MOSPD2) is a protein recently described as expressed on monocytes and neutrophils and as regulating their migration regardless of the activating chemokine [5, 6]. In a mouse model of multiple sclerosis (MS), MOSPD2 deficiency or treatment with anti-MOSPD2 monoclonal antibodies (mAbs) protected against disease development and restricted infiltration of monocytes into the CNS [7]. However, the exact mechanism by which MOSPD2 controls monocyte migration was unknown. In this study, a possible role for MOSPD2 in regulating integrin activation and adhesion in monocytes was explored. The results show, for the first time, that in monocytes, MOSPD2 binds β2-integrin to control its state of activation, demonstrating a role in regulating adhesion and release of monocytes through β2 integrin from the ECM and from adhesion molecules. Moreover, targeting MOSPD2 with a humanized anti-MOSPD2 mAb IW- 601 has the potential to treat inflammatory diseases by inhibiting monocyte infiltration into inflamed tissues.

## Materials and methods

### Control and anti-MOSPD2 mAb

To generate a humanized anti-human MOSPD2 mAb, mice were first immunized with the extracellular region of human MOSPD2. Next, hybridoma supernatants were screened by ELISA and flow cytometry for binding to human MOSPD2. One positive clone was then further developed to become a humanized anti-MOSPD2 antibody named IW- 601. IW- 601 was produced by transient transfection of the heavy and light chains into proprietary Xten-CHO cells (ProteoGenix, France) (< 1 EU/mg). Human IgG1 isotype control antibody (< 1 EU/mg) was purchased from BioXcell.

### Isolation of human immune cell subsets

Peripheral blood mononuclear cells (PBMCs) were isolated on Lymphoprep (Alera Technologies, Norway) using 50-ml Leucosep tubes (Greiner Bio-One, Germany). Cells were washed in PBS (Beit Haemek, Israel), and incubated at 4 °C for 15 min in a buffer containing PBS and 0.5% BSA with human CD14 or Pan T cell isolation kit microbeads (Miltenyi Biotec).

### Lentiviral transduction and transient transfection

To silence protein expression, THP- 1 cells ($2 \times 10^6$ in 2 ml) were placed in a 15-ml tube, and CRISPR-CAS9 (hereafter CRISPR) non-target control (CRISPR-Control) (CRISPR12 V, Sigma), CRISPR human MOSPD2 (CRISPR-MOSPD2) (HSPD0000142520, Sigma), or CRISPR human β2-integrin (CRISPR-β2-integrin) (HSPD0000021931, Sigma) lentiviral particles were applied on the cells. Cells were centrifuged for 60 min at 2000 rpm at RT in the presence of 8 µg/ml polybrene (Sigma) and were seeded in a 6-well plate. After 72 h, fresh medium containing puromycin (4 µg/ml Sigma) was added for the selection of transduced cells. Single-cell cloning was performed on CRISPR-transduced cells to isolate cells with silenced protein expression. For stable expression, HEK 293 cells ($2 \times 10^6$ in 2 ml) were transduced with lentiviral particles expressing HA-tagged full-length human MOSPD2 for 60 min at 2000 rpm at RT in the presence of 8 µg/ml polybrene (Sigma). The cells were then seeded in a 6-well plate. After 72 h, fresh medium containing puromycin (4 µg/ml Sigma) was added for the selection of transduced cells. For transient expression, HEK 293 cells ($2 \times 10^6$ in 2 ml) were transfected for 48 h using jetPRIME transfection reagent (Polyplus-transfection, France) with plasmids

expressing FLAG-tagged β1-integrin or β2-integrin (Origene, Rockville, MD, USA). Transfection efficiency was determined by flow cytometry gating on live cells.

## Western blots

Cells were washed and resuspended in lysis buffer containing 1:100 dithiothreitol (DTT) and phosphatase and protease inhibitors (Thermo Scientific). Samples were loaded onto a precast Criterion TGX gel (Bio-Rad, Hemel Hempstead, UK) and transferred onto a nitrocellulose membrane. Blots were blocked with 5% milk or BSA in Tris-buffered saline and Tween 20 (TBST) for 1 h, followed by incubation with primary and secondary antibodies. Membranes were developed using Immobilon® Forte western blot HRP substrate (Millipore). The following antibodies were used for immunoblotting:

*Primary antibodies*: Phospho extracellular-regulated kinase (pERK)1/2 was purchased from Sigma (Israel). Heat shock protein (HSP) 90 and C-YES were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Phospho-focal adhesion kinase (p-FAK Y397) and p-AKT were from Cell Signaling Technology (Beverly, MA, USA). Phospho-FAK (Y407) was from Abcam (Cambridge, UK), and mouse anti-MOSPD2 mAb was developed in-house.

*Secondary antibodies*: HRP donkey anti-rabbit (1:5000) and HRP goat anti-mouse (1:3000) were from Jackson ImmunoResearch (West Grove, PA, USA).

## Cell migration and adhesion

*Migration*: MCP- 1 (CCL2, 100 ng/ml) and SDF- 1 (CXCL12, 100 ng/ml) (PeproTech, Israel) were dissolved in 0.5% FBS/RPMI- 1640 and placed in the lower chamber of a QCM 24-well migration assay plate (5 μm pores) (Corning-Costar, Corning, NY). CRISPR-treated THP- 1 cells or human primary CD14 + monocytes were seeded ($3 \times 10^5$) in the upper chamber, followed by incubation for 3 h, after which the number of cells that migrated to the lower chamber was determined using flow cytometry. CD14 + monocytes were incubated with IW- 601 or an isotype control antibody for 30 min before loading onto the upper chamber.

*Adhesion*: Adhesion assays to ECM molecules were performed on pre-coated plates (Cell Biolabs, Inc). To bind cells to adhesion molecules, 48-well plates were coated overnight at 4 °C with ICAM- 1 or VCAM- 1 (10 μg/ml) (PeproTech, Israel). Cells were suspended in 0.5% FBS RPMI medium and seeded ($1 \times 10^6$/well) on coated plates for 90 min at 37 °C. The media were then aspirated, and wells were washed with PBS three times. Cells were fixed, stained with cell stain solution for 10 min, and washed five times, and the wells were left to air dry. Then, 200 μl of extraction solution were added per well, and the plate was incubated for 10 min on an orbital shaker. Finally, 150 μl from each extracted sample were transferred to a 96-well microtiter plate, and the plate was read with a 560-nm filter on a plate reader. CD14 + monocytes were incubated with IW- 601 or an isotype control antibody for 40 min prior to seeding onto adhesion plates.

## Flow cytometry staining

To stain for high-affinity αLβ2, cells were suspended in 200 μl of medium containing 0.5% FCS in RPMI, transferred to FACS tubes, and stained with anti-human LFA- 1 Alexa-Fluor 647 clone m24 (BioLegend) for 30 min at 37 °C. Where indicated, mouse IgG1 Alexa-Fluor 647 was used as a control stain. After that, cells were washed with FACS buffer (PBS + 2%FCS + 0.02% $NaN_3$), centrifuged, re-suspended with 200 μl FACS buffer, and analyzed. Staining with integrin-β2-PE (clone TS1/18) and isotype control-PE antibody (BioLegend) was done in FACS buffer for 30 min at 4 °C. FACSCalibur or NovoCyte flow cytometers were used for sample acquisition and analysis.

## Immunoprecipitation

Cells were lysed using a 1% Brij- 98 lysis buffer containing protease inhibitors, followed by a 20-min incubation on ice and a 15-min centrifugation at maximum speed. Samples were incubated in a rotator overnight at 4 °C with anti-HA (clone 3 F10) or anti-FLAG (clone M2). Protein A/G beads (Santa Cruz) were added for 2 h. Protein elution was performed with loading buffer containing 62.5 mM Tris, 2.3% SDS, 10% glycerol, and 0.02% pyronin for 10 min at boiling temperature.

## Patients and healthy controls

### MS

Eligibility criteria included men and women > 18 years of age and a diagnosis of relapsing–remitting MS, primary progressive MS, or secondary progressive MS. Exclusion criteria were pregnancy and nursing women, other chronic and systemic autoimmune diseases, other immunosuppressive or immunomodulatory therapies given for other immune-mediated diseases, and active malignant disease in the last 5 years prior to sampling.

### RA and PsA

Eligibility criteria included men and women > 18 years of age and a confirmed diagnosis of rheumatoid arthritis (RA) according to the 2010 criteria of the American College of Rheumatology for RA [8] or psoriatic arthritis (PsA) as

confirmed by the classification of psoriatic arthritis criteria [9]. Exclusion criteria were pregnancy and nursing women, other chronic and systemic autoimmune diseases, and active malignant disease in the last 5 years prior to sampling.

### UC and CD

Eligibility criteria included men and women > 18 years of age, a confirmed diagnosis of ulcerative colitis (UC) and active disease as indicated by clinical Mayo score ≥ 2 or a confirmed diagnosis of Crohn's disease (CD) involving the small intestine and/or colon, and active disease as indicated by Crohn's Disease Activity Index (CDAI) ≥ 150. Exclusion criteria were pregnancy and nursing women, sclerosis, other chronic and systemic autoimmune diseases, other immunosuppressive/immunomodulatory therapies given for other immune-mediated diseases, and active malignant disease in the last 5 years prior to sampling.

### Healthy controls

Healthy controls ($n = 11$) included men and women > 18 years of age.

## In vivo mouse models

Collagen-induced arthritis (CIA): for the induction of CIA, 14–17-week-old male DBA/1 mice (The Jackson Laboratory) were injected subcutaneously in the flank with 0.1 mL emulsion containing CFA (2 mg/mL, Difco) and 1 mg/mL Bovine Type II Collagen (C-II, MD Biosciences) on day 0 and a second injection on day 21 into the flank with 0.1 mL emulsion containing 1 mg/mL C-II in IFA. With the first clinical manifestation (day 22), mice were divided into groups and treatment began with isotype control Ab (BioX-Cell BP0083; 0.5 mg), Anti-TNF-α (BioXCell BP0058; 0.2 mg), or Anti-MOSPD2 mAb (0.5 mg) every 3–4 days intraperitoneally up to day 40. For the arthritis score, each paw was scored on a scale of 0–4 for the degree of swelling, erythema, and deformity of the joints (maximum score 16 per animal) as follows: 0 = normal, 1 = slight erythema and/or swelling of the ankle or wrist, 2 = moderate erythema and/or swelling of the ankle or wrist, 3 = severe erythema and/or swelling of the ankle or wrist, 4 = complete erythema and swelling of toes or fingers and ankle or wrist and inability to bend the ankle or wrist. Paw swelling was measured using a caliper.

Trinitro benzenesulfonic acid (TNBS)-induced colitis: for the induction of colitis, 24 h after fasting (day 0), 6–8-week-old female Balb/c mice (Envigo) were anesthetized via intraperitoneal injection of 3% sodium pentobarbital and, using a 21G thin polyurethane catheter, injected rectally with 100 µl of 1.4% TNBS solution dissolved in 50% ethanol. Two days prior to disease induction and on experimental days 0 and 3, mice were administered intraperitoneally with 0.5 mg of anti-MOSPD2 or isotype control antibody. Colitis severity, represented by the disease activity index (DAI), was assessed according to supplemental Table 1. For cytokine production, upon sacrifice, colon explants were placed for 24 h in RPMI- 1640 containing 10% FCS, after which supernatants were collected and analyzed by ELISA.

**Table 1** Efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from healthy controls (HC)

| Code | Age | Sex | Mean number of migrating cells[a] ± S.D. (% inhibition of migration) | |
|---|---|---|---|---|
| | | | Control IgG | IW- 601 |
| HC- 1 | 41 | F | 9456 ± 188 | 407 ± 97 (96)*** |
| HC- 2 | 57 | M | 7623 ± 466 | 584 ± 56 (92)*** |
| HC- 3 | 37 | F | 5174 ± 559 | 364 ± 171 (93)*** |
| HC- 4 | 35 | F | 27,770 ± 9143 | 1791 ± 211 (94)** |
| HC- 5 | 27 | M | 5541 ± 854 | 1518 ± 398 (73)** |
| HC- 6 | 47 | F | 3177 ± 913 | 796 ± 200 (75)* |
| HC- 7 | 28 | M | 724 ± 386 | 54 ± 18 (93) |
| HC- 8 | 42 | M | 1365 ± 462 | 471 ± 333 (65) |
| HC- 9 | 24 | M | 9402 ± 3647 | 1086 ± 121 (88)* |
| HC- 10 | 42 | M | 19,750 ± 237 | 2265 ± 475 (89)*** |

[a]Results are the means of triplicate runs

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with human control IgG

## Statistics

Statistical analysis were performed using SigmaPlot 14.0 (SigmaPlot Software). Statistical significance of differences between groups in Tables 1, 2, 3, 4, 5, and 6 was determined by 2-tailed Student's *t* test, and data are expressed as mean ± S.D. For in vivo studies, differences between groups were determined by ANOVA with multiple comparison test. The criterion for statistical significance was a *p* value of less than 0.05.

## Study approval

All animal experiments described in this paper were approved by the Institutional Animal Care and Use Committee of the Sheba Medical Center, Ramat Gan, Israel. Venous blood samples were drawn from patients in compliance with the Institutional Review Board at the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. Signed informed consent was received from all participants prior to sample donation.

**Table 2** Clinical characteristics and efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from RRMS patients

| Code | Age | Sex | EDSS | Active therapy | Mean number of migrating cells[a] ± S.D. (% inhibition of migration) | |
|---|---|---|---|---|---|---|
| | | | | | Control IgG | IW- 601 |
| MS- 1 | 62 | M | 5 | Cladribine | 6617 ± 601 | 1802 ± 182 (73)*** |
| MS- 2 | 30 | F | 6 | Cladribine | 5074 ± 891 | 1142 ± 168 77** |
| MS- 3 | 50 | M | 1 | DMF[b] | 15,550 ± 2,301 | 11,388 ± 212 (27)* |
| MS- 4 | 55 | M | 1 | Teriflunomide | 5664 ± 1075 | 515 ± 243 (91)* |
| MS- 5 | 57 | M | 3.5 | Interferon beta- 1a | 4382 ± 176 | 527 ± 88 (88)*** |
| MS- 6 | 53 | F | 2 | Natalizumab | 15,845 ± 304 | 5108 ± 947 (68)*** |
| MS- 7 | 35 | F | 0 | DMF | 8269 ± 1061 | 3798 ± 550 (54)** |
| MS- 8 | 28 | F | 0 | DMF | 13,706 ± 570 | 1733 ± 338 (87)*** |
| MS- 9 | 26 | F | 0 | DMF | 21,187 ± 2344 | 5932 ± 1383 (72)*** |
| MS- 10 | 35 | F | 2.5 | DMF | 2647 ± 262 | 733 ± 158 (72)*** |
| MS- 11 | 27 | F | 3.5 | Ocrelizumab | 6781 ± 1205 | 583 ± 257 (91)*** |
| MS- 12 | 44 | F | 6 | Interferon beta- 1a | 285 ± 162 | 16 ± 6 (95)* |
| MS- 13 | 51 | F | 2 | DMF | 783 ± 6 | 125 ± 9 (84)** |
| MS- 14 | 22 | M | 0 | DMF | 6036 ± 1715 | 1586 ± 121 (74)* |
| MS- 15 | 32 | M | 0 | DMF | 16,986 ± 5282 | 2349 ± 544 (86)** |
| MS- 16 | 34 | M | 2 | DMF | 17,742 ± 2519 | 4565 ± 1675 (74)** |
| MS- 17 | 24 | F | 1 | DMF | 5115 ± 556 | 221 ± 85 (96)*** |
| MS- 18 | 26 | F | 0 | DMF | 7328 ± 644 | 5172 ± 776 (29)* |
| MS- 10 | 45 | M | 1 | DMF | 7579 ± 1700 | 1875 ± 182 (75)* |
| MS- 20 | 66 | F | 5.5 | Ocrelizumab | 12,854 ± 1611 | 1505 ± 290 (88)*** |
| MS- 21 | 45 | F | 1 | Interferon beta- 1a | 5670 ± 408 | 761 ± 44 (87)*** |
| MS- 22 | 26 | F | 1 | None | 6879 ± 803 | 1511 ± 335 (78)*** |
| MS- 23 | 47 | F | 3 | Cladribine | 4044 ± 557 | 404 ± 182 (90)*** |
| MS- 24 | 60 | F | 4.5 | DMF | 17,102 ± 84 | 1562 ± 138 (91)*** |

[a]Results are the mean of triplicate runs

[b]Dimethyl fumarate

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with human control IgG

**Table 3** Clinical characteristics and efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from PPMS and SPMS patients

| Code | Age | Sex | Diagnosis | EDSS | Active therapy | Mean number of migrating cells[a] ± S.D. (% inhibition of migration) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Control IgG | IW- 601 |
| MS- 26 | 66 | F | PPMS | 6 | Ocrelizumab | 3058 ± 420 | 711 ± 333 (77)** |
| MS- 27 | 62 | M | PPMS | 6.5 | Ocrelizumab | 11,772 ± 2,418 | 3698 ± 84 (69)** |
| MS- 28 | 56 | F | SPMS | 6 | Interferon beta- 1a | 3250 ± 955 | 335 ± 101 (90)** |
| MS- 29 | 60 | F | SPMS | 6.5 | Ocrelizumab | 1272 ± 289 | 37 ± 19 (97)** |
| MS- 30 | 61 | M | PPMS | 6.5 | Ocrelizumab | 18,916 ± 3052 | 979 ± 204 (95)*** |
| MS- 31 | 54 | F | SPMS | 6 | Cladribine | 2000 ± 460 | 217 ± 86 (89)** |
| MS- 32 | 39 | M | PPMS | 5.5 | Glatiramer acetate | 5585 ± 210 | 496 ± 61 (91)*** |
| MS- 33 | 56 | F | SPMS | 6 | Ocrelizumab | 5592 ± 1210 | 771 ± 415 (86)** |

[a]Results are the means of triplicate runs

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with human control IgG

**Table 4** Clinical characteristics and efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from RA patients

| Code | Age | Sex | CDAI | Active therapy | Mean number of migrating cells[a] ± S.D. (% inhibition of migration) | |
|---|---|---|---|---|---|---|
| | | | | | Control IgG | IW- 601 |
| RA- 1 | 83 | F | 2 | Methotrexate, baricitinib | 7957 ± 381 | 2141 ± 272 (73)*** |
| RA- 2 | 64 | M | 9 | Methotrexate, hydroxychloroquine | 1074 ± 98 | 163 ± 43 (85)*** |
| RA- 3 | 65 | F | 0 | Methotrexate, baricitinib | 3488 ± 599 | 343 ± 139 (90)*** |
| RA- 4 | 75 | F | 15 | Baricitinib, prednisone | 7037 ± 1,606 | 682 ± 1 (90)* |
| RA- 5 | 68 | M | 33 | Methotrexate, prednisone | 5520 ± 1,827 | 653 ± 179 (88)* |
| RA- 6 | 79 | F | 37 | None | 23,766 ± 7,090 | 2642 ± 149 (89)** |
| RA- 7 | 72 | F | 48 | Methotrexate, prednisone | 1818 ± 769 | 228 ± 23 (87)* |
| RA- 8 | 60 | F | 27 | Methotrexate, hydroxychloroquine | 3463 ± 1,839 | 337 ± 198 (90)* |
| RA- 9 | 41 | F | 3 | Methotrexate, prednisone | 9934 ± 3,454 | 1366 ± 236 (86)* |
| RA- 10 | 60 | M | 0 | Methotrexate | 3307 ± 593 | 1135 ± 318 (66)** |
| RA- 11 | 55 | M | 5 | Methotrexate, etanercept | 2488 ± 36 | 472 ± 142 (81)*** |
| RA- 12 | 51 | F | 22 | Methotrexate, sulfasalazine | 1890 ± 57 | 354 ± 73 (81)*** |

[a]Results are the mean of triplicate runs

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control IgG

**Table 5** Clinical characteristics and efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from PsA patients

| Code | Age | Sex | DAPSA/PASI | Active therapy | Mean number of migrating cells[a] ± S.D. (% inhibition of migration) | |
|---|---|---|---|---|---|---|
| | | | | | Control IgG | IW- 601 |
| PsA- 1 | 46 | M | 9.4/7.2 | Methotrexate, infliximab | 4702 ± 1,431 | 1118 ± 632 (76)* |
| PsA- 2 | 46 | M | N.D/0 | Adalimumab | 5010 ± 336 | 535 ± 20 (89)*** |
| PsA- 3 | 51 | F | 7.6/0 | Adalimumab | 2267 ± 218 | 318 ± 254 (86)*** |
| PsA- 4 | 55 | M | 28/5.6 | Ixekizumab | 4253 ± 967 | 497 ± 182 (88)** |
| PsA- 5 | 69 | F | N.D/2.8 | Adalimumab | 19,332 ± 4953 | 9313 ± 4589 (52) |
| PsA- 6 | 62 | F | 12.9/0 | Infliximab | 5442 ± 627 | 359 ± 118 (93)*** |
| PsA- 7 | 54 | F | 0/0 | Etanercept | 2763 ± 491 | 615 ± 211 (78)** |

[a]Results are the means of triplicate runs

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control IgG

## Results

### MOSPD2 regulates monocyte adhesion

To assess the effect of MOSPD2 on monocyte adhesion, we initially silenced protein expression in THP- 1 cells using CRISPR-CAS9 technology. Protein silencing was verified by western blots (Fig. 1A). The ability of THP- 1 clones silenced for the expression of MOSPD2 to migrate in a migration assay was decreased by more than 75% compared with CRISPR-control cells (Fig. 1B). However, when seeded on plates coated with type IV collagen, a prominent ECM compound, clones lacking MOSPD2 demonstrated an up to twofold increase in adhesion compared with CRISPR-control cells (Fig. 1C). The increased adhesion of MOSPD2-silenced clones was not restricted to collagen IV and was also demonstrated for other ECM components (Fig. 1D).

Recent reports suggest that MOSPD2 can also be located intracellularly in certain types of cells [10, 11]. To test whether the effect on monocyte adhesion can be attributed to surface-expressed MOSPD2, we developed IW- 601, a humanized mAb against MOSPD2. The results in Fig. 2A show that IW- 601 inhibits monocyte migration in a dose-dependent manner. In the presence of IW- 601, the adhesion of monocytes to various ECM ligands and to adhesion molecules was increased, also in a dose-dependent manner (Fig. 2B–D).

### MOSPD2 governs αLβ2 conformational state

Integrins play a principal role in the adhesion of monocytes to the vascular endothelium and ECM. Integrin αLβ2 is one of the most frequently investigated integrins with regard to structural alterations following activation.

**Table 6** Clinical characteristics and efficacy of IW- 601 inhibitory effect on the migration of monocytes isolated from colitis patients

| Code | Age | Sex | Diagnosis | CDAI/Mayo score | Active therapy | Mean number of migrating cells[a] ± S.D (% inhibition of migration) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Control IgG | IW- 601 |
| IBD- 1 | 38 | M | CD | 152 | None | 17,346 ± 5567 | 646 ± 62 (96)** |
| IBD- 2 | 33 | F | CD | 165 | Infliximab | 8289 ± 3710 | 323 ± 122 (96)* |
| IBD- 3 | 70 | F | CD | 277 | Vedolizumab | 13,210 ± 2900 | 1585 ± 239 (88)** |
| IBD- 4 | 58 | F | CD | 208 | Vedolizumab, azathioprine | 10,414 ± 2996 | 1146 ± 250 (89)** |
| IBD- 5 | 25 | M | CD | 174 | Adalimumab | 39,432 ± 3873 | 7535 ± 2290 (81)*** |
| IBD- 6 | 27 | F | CD | 278 | Infliximab | 17,944 ± 4001 | 9196 ± 1510 (49) |
| IBD- 7 | 56 | F | CD | 192 | Infliximab | 53,553 ± 8882 | 14,748 ± 830 (72) |
| IBD- 8 | 66 | F | UC | 3 | Vedolizumab | 5568 ± 509 | 1783 ± 402 (68)*** |
| IBD- 9 | 56 | F | UC | 3 | Vedolizumab, mesalazine | 10,467 ± 1489 | 893 ± 114 (91)*** |
| IBD- 10 | 33 | F | UC | 6 | Infliximab | 3354 ± 690 | 613 ± 103 (82)** |
| IBD- 11 | 22 | M | UC | 3 | Vedolizumab, mesalazine | 13,090 ± 4042 | 1126 ± 399 (91)** |
| IBD- 12 | 66 | F | UC | 3 | Vedolizumab, mesalazine | 23,819 ± 4631 | 3940 ± 584 (83)** |

[a]Results are the means of triplicate runs

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control IgG

These studies were facilitated in part by flow cytometry staining using m24, an antibody clone that specifically recognizes human αLβ2 in its high-affinity extended-open conformation [12, 13]. To test for a possible role for MOSPD2 in controlling the activation state of integrins, MOSPD2-silenced THP- 1 clones were stained with the m24 antibody. As depicted in Fig. 3A, THP- 1 clones in which MOSPD2 was silenced displayed increased staining for high-affinity αLβ2 compared with CRISPR-control cells. Treatment of THP- 1 wild-type cells with IW-601 induced the transition of αLβ2 into the high-affinity state in a dose-dependent manner (Fig. 3B), which was sustained for over 4 h (Fig. 3C). When applied to primary cells, IW- 601 induced the transition of αLβ2 to the high-affinity state in peripheral blood monocytes, but not in T cells, which do not express MOSPD2 (Fig. 3D–E). Surface expression of β2-integrin was not apparently altered in MOSPD2-silenced or IW- 601-treated cells (Supp. Figs. S1 A-B). Moreover, IW- 601 did not induce the expression of surface markers typically upregulated on activated monocytes (Supp. Figure 2), indicating the specificity of its effect on integrins.

Activation and binding of high-affinity integrins to extracellular ligands are associated with the induction of intracellular signaling pathways. We, therefore, sought to test whether interfering with MOSPD2 will affect phosphorylation events. Indeed, in monocytes treated with IW- 601, signaling in the FAK, AKT, and ERK pathways, which are associated with integrin activation, was found to be induced (Fig. 3F-G).

## MOSPD2 binds β2-integrin but not β1-integrin

Since the unclasping of the integrin heterodimer to the extended-open conformation is predominantly driven by changes in the tilt angle of the β-subunit [14, 15], we hypothesized that MOSPD2 may bind directly to the β-subunit, thus regulating integrin conformation. To test that, HEK293 cells stably expressing HA-tagged MOSPD2 were transiently transfected with FLAG-tagged β2-integrin, followed by co-immunoprecipitation. The data shown in Fig. 4A demonstrate that MOSPD2 precipitates along with β2-integrin. Nevertheless, attempts to co-immunoprecipitate MOSPD2 with β1-integrin resulted in a negative outcome (Fig. 4B). To gain a perspective on the scale of influence that MOSPD2 has on monocyte adhesion and migration in light of the key role β2-integrins have on cell motility, we silenced the expression of β2-integrin in THP- 1 cells (Supp. Fig. S3) and compared their migration and adhesion with that of MOSPD2-silenced cells. The results presented in Fig. 4C show that cells silenced for either MOSPD2 or β2-integrin undergo restricted migration. Nonetheless, unlike MOSPD2-silenced cells, which show higher adhesion, ablation of β2-integrin led to reduced cell adhesion (Fig. 4D).

## MOSPD2 is a potential therapeutic target for the treatment of inflammatory diseases

There is a line of evidence indicating that monocytes are strongly associated with the pathogenesis of various autoimmune inflammatory diseases [16–20]. In light of the

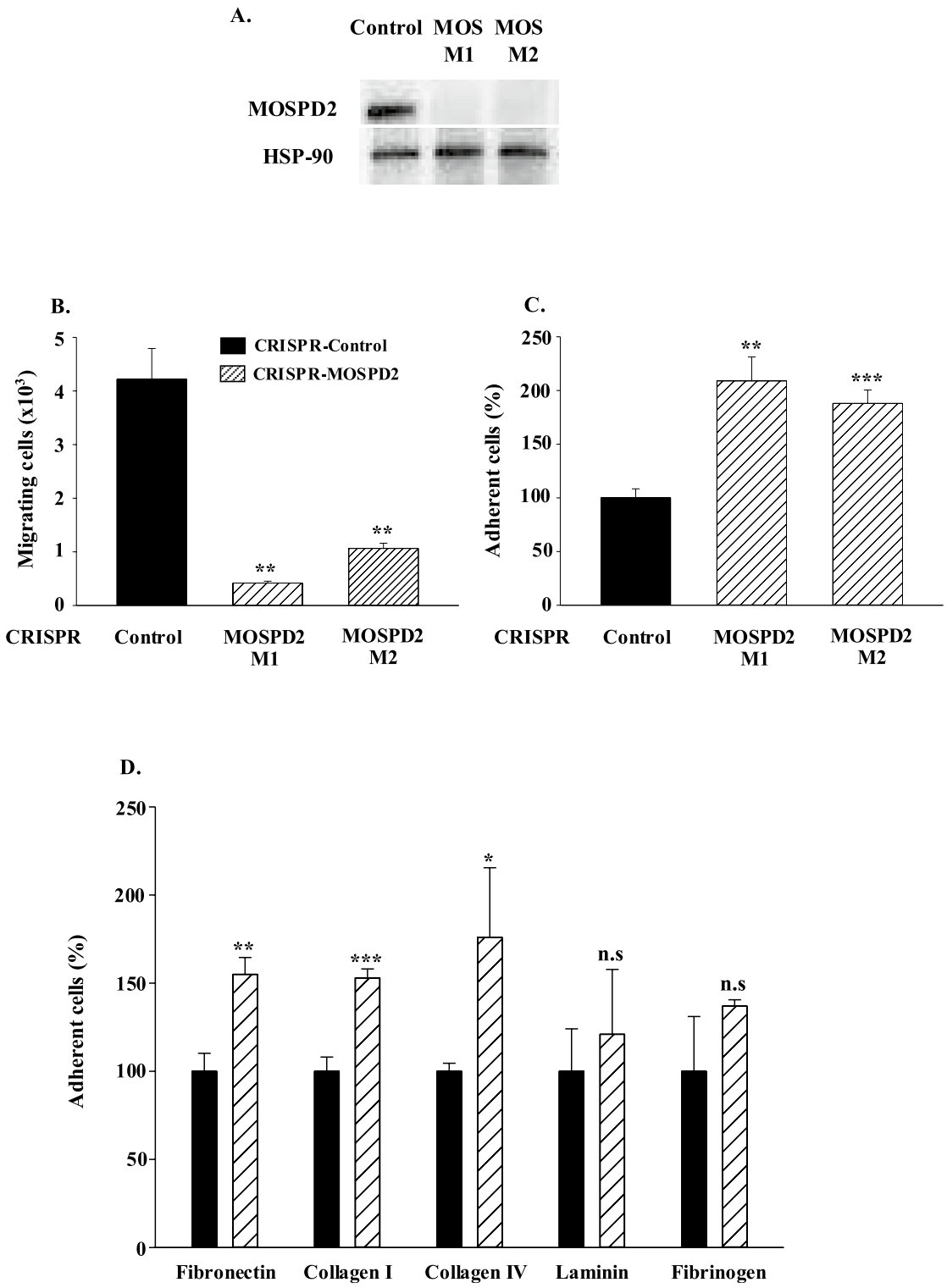



**Fig. 1** MOSPD2 regulates monocyte adhesion. **A** Protein expression of MOSPD2 in THP- 1 cells transduced with CRISPR-control or CRISPR-MOSPD2 lentiviral particles. Two different isolated clones (M1, M2) are shown for the CRISPR-MOSPD2 cells. HSP- 90 was used as a loading control. **B** Trans-well migration of THP- 1 cells described in **A** toward SDF- 1 and MCP- 1 (100 ng/ml). Cell count is presented. Samples were run in triplicate. Mean ± S.D. is shown. **C** Adhesion of cells described in **A** on a plate coated with type IV collagen. Relative adhesion based on OD to CRISPR-control is presented. Samples were run in triplicate. Mean ± S.D. is shown. **D** Adhesion of THP- 1 cells transduced with CRISPR-control and the M1 MOSPD2-silenced clone seeded onto a plate coated with different ECM ligands. Relative adhesion based on OD to CRISPR-control is presented. Samples were run in triplicate. Mean ± S.D. is shown. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

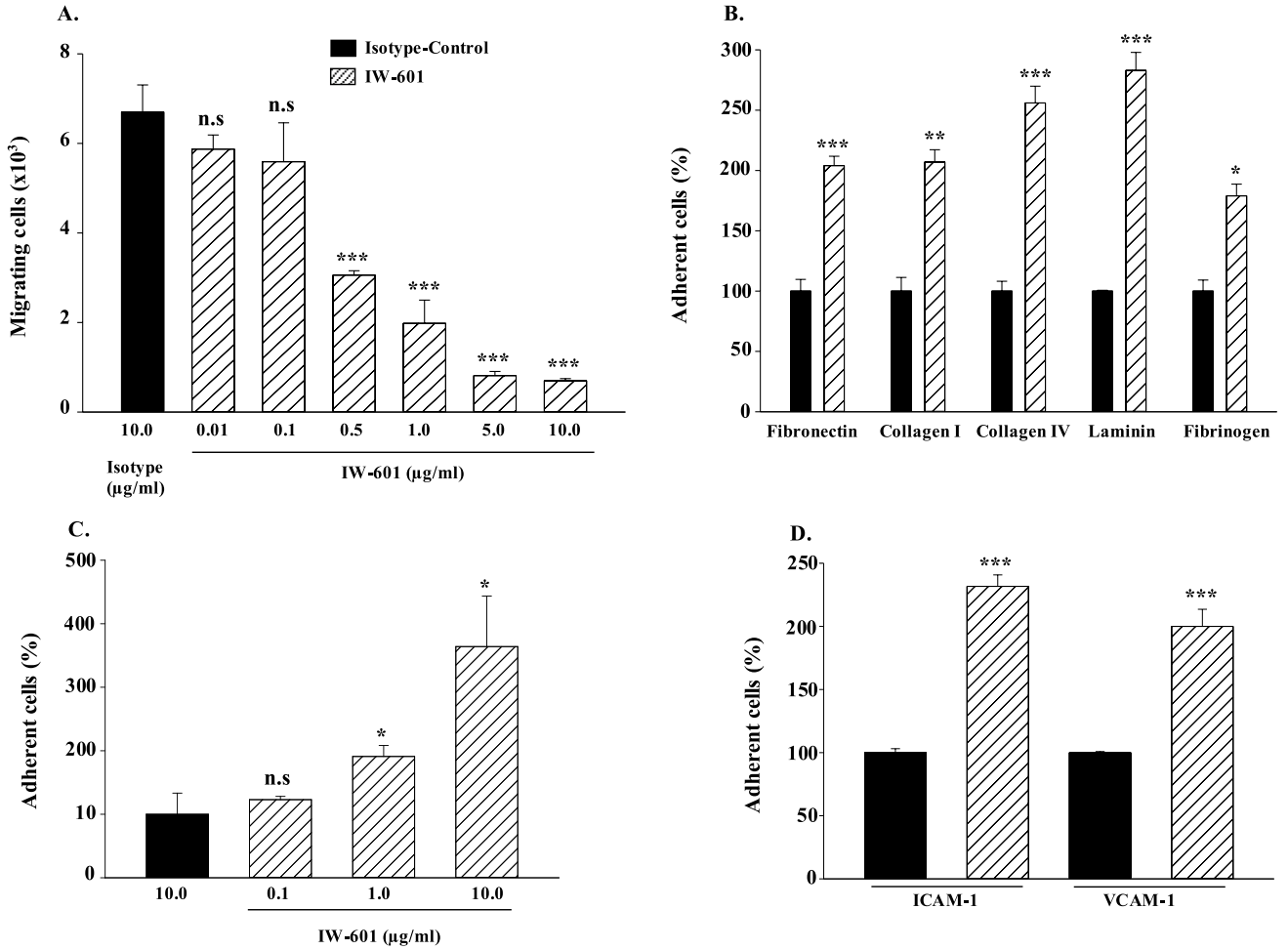
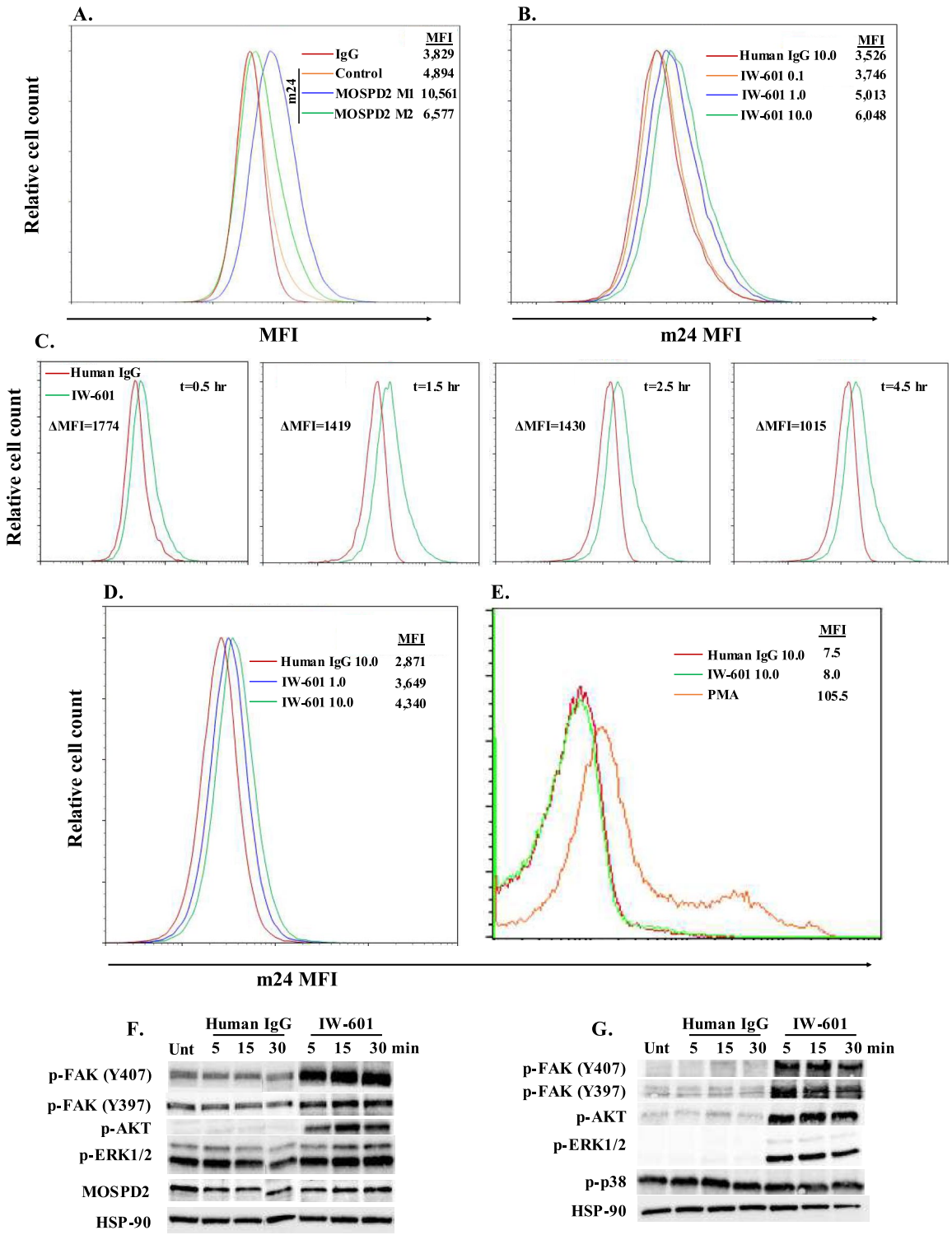



**Fig. 2** IW- 601, a humanized anti-MOSPD2 antibody, enhances the adhesion of monocytes. **A** Dose-dependent effect of IW- 601 on the migration of human primary monocytes toward SDF- 1 and MCP- 1 (100 ng/ml). Cell count is presented. Samples were run in triplicate. Mean ± S.D. is shown. **B** Adhesion of human primary monocytes treated with human isotype control antibody or IW- 601 (10 µg/ml) to plates coated with different ECM ligands. Relative adhesion based on OD to human isotype control–treated cells is presented. Samples were run in triplicate. Mean ± S.D. is shown. **C** Adhesion of human primary monocytes treated with human isotype control antibody (10 µg/ml) or different doses of IW- 601 on a plate coated with fibronectin. Relative adhesion based on OD to isotype control–treated cells is presented. Samples were run in triplicate. Mean ± S.D. is shown. **D** Adhesion of human primary monocytes treated with human isotype control antibody or IW- 601 (10 µg/ml) to a plate coated with ICAM-1 or VCAM- 1. Relative adhesion based on OD to isotype control–treated cells is presented. Samples were run in triplicate. Mean ± S.D. is shown

strong in vitro effect of IW- 601 on adhesion and migration in primary monocytes from healthy donors, we sought to assess the potential of IW- 601 to inhibit monocyte migration in patients with various chronic inflammatory diseases. As expected, monocytes isolated from healthy controls ($n = 10$) that were treated ex vivo with IW- 601 showed significantly inhibited migration (Table 1). In parallel, we isolated monocytes from the peripheral blood of MS, RA, PsA, UC, and CD patients and tested their migration in the presence of IW- 601 or an IgG control antibody. For MS, 24 samples were collected from relapsing remitting MS (RRMS) patients, 4 samples were from primary progressive MS (PPMS) patients, and 4 were from secondary progressive MS (SPMS) patients. RRMS patients were aged 22–66 (median 39.5), were mostly females (16/24—67%), and had Expanded Disability Status Scale (EDSS) scores in the range 0–6. At the time of sampling, one patient was not undergoing treatment (MS- 22), and the rest were being treated with various medications, including dimethyl fumarate (DMF, 13/24—54%), natalizumab (1/24—4%), cladribine (3/24—12%), ocrelizumab (2/24—8%), teriflunomide (1/24—4%), and interferon-beta (3/24—12%). Progressive patients were aged 39–66 (median 58), were mostly females (5/8—62.5%), and had EDSS scores in the range 5.5–6.5. At the time of sampling, progressive patients were being treated with ocrelizumab (5/8—62.5%), interferon-beta (1/8—12.5%), cladribine (1/8—12.5%), and glatiramer acetate (1/8—12.5%). Tables 2 and 3 show the clinical characteristics of the MS




patients and the efficacy of IW- 601 in blocking ex vivo migration of monocytes isolated from these patients.

For the arthritis patients, 12 samples were collected from RA patients and 7 samples were collected from PsA patients. RA patients were aged 41–83 (median 64.5) and were mostly females (8/12—67%). Based on the calculated Clinical Disease Activity Index (CDAI) score, three RA patients were in remission (3/12—25%; CDAI 0–2.8), three exhibited low disease activity (3/12—25%; CDAI 3–10), and two had moderate disease activity (2/12—17%; CDAI 10.1–22). The remaining four RA patients had high disease activity (4/12—33%, CDAI 22.1–76). At the time of sampling, the

◂**Fig. 3** MOSPD2 governs the conformational state of αLβ2. **A** THP- 1 CRISP-control or CRISPR-MOSPD2 clones (M1, M2) were stained with anti-αLβ2 clone m24. CRISPR-control cells were also stained with an isotype control antibody (IgG). **B** THP- 1 cells were treated with human isotype control antibody (10 μg/ml) or different doses of IW- 601 (0.1–10 μg/ml) and stained for 30 min with anti-αLβ2 clone m24. **C** THP- 1 cells were treated with human isotype control antibody or IW- 601 (10 μg/ml) for the indicated times and stained with anti-αLβ2 clone m24 for the last 30 min of treatment. **D** Human primary monocytes were treated with human isotype control antibody (10 μg/ml) or different doses of IW- 601 (1 and 10 μg/ml) and stained for 30 min with anti-αLβ2 clone m24. **E** Human primary T cells were treated with human isotype control antibody (10 μg/ml), IW- 601 (10 μg/ml) or PMA (5 ng/ml) as a positive control and stained for 30 min with anti-αLβ2 clone m24. **F** THP- 1 or **G** human primary monocytes were seeded onto a plate coated with fibronectin for 60 min, after which human isotype control antibody or IW- 601 (10 μg/ml) were added for the indicated times. Blots were stained to demonstrate the induction of signaling events. HSP- 90 and C-YES were used as loading controls for THP- 1 and human primary monocytes, respectively

RA patients were either untreated (RA- 6), receiving monotherapy of methotrexate (RA- 10), or being treated with combinations of various disease-modifying antirheumatic drugs including methotrexate (10/12—83%), baricitinib (2/12—17%), hydroxychloroquine (2/12—17%), prednisone (4/12—33%), etanercept (1/12—8%), and sulfasalazine (1–12—8%). PsA patients were aged 46–69 (median 54) and were mostly females (4/7—57%). Disease activity in psoriatic arthritis (DAPSA) scores could be determined in only five patients and indicated that one patient (PsA- 7) was in remission and four exhibited low to moderate disease activity. Psoriasis Area and Severity Index (PASI) scores ranged from 0 (4/7—57%) to 7.2. At the time of sampling, most PsA patients were being treated with TNFα antagonists (6/7—86%). One patient (PsA- 4) was being treated with an anti-IL- 17 antibody and one was being treated with a TNFα-blocker in combination with methotrexate (PsA- 1). Tables 4 and 5 show the clinical characteristics of the RA and PsA patients and the efficacy of IW- 601 in blocking ex vivo migration of their peripheral blood monocytes.

For the colitis patients, 7 samples were from patients diagnosed with CD, and 5 were from patients diagnosed

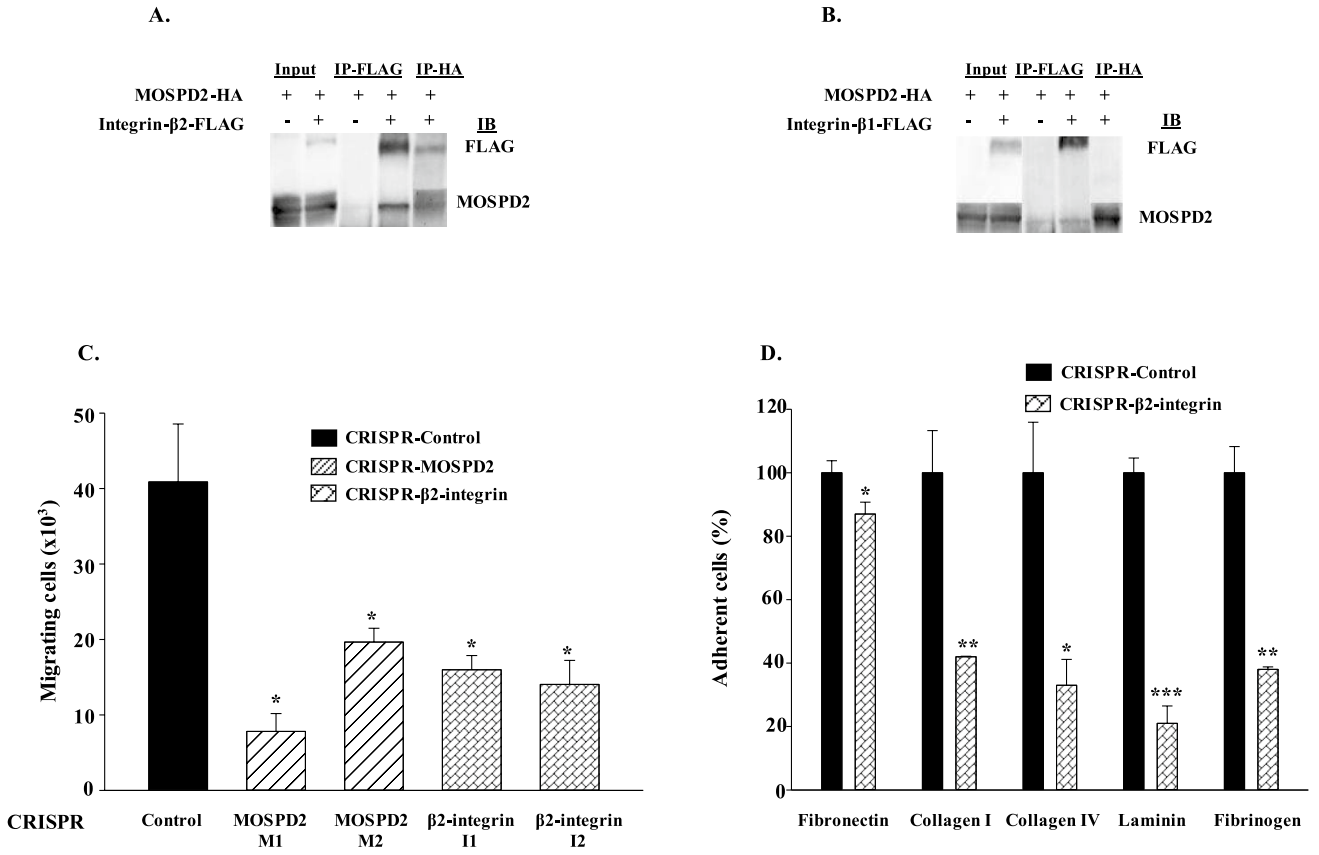



**Fig. 4** MOSPD2 binds β2-integrin. **A**–**B** HEK 293 cells stably expressing HA-tagged MOSPD2 were left untouched or transiently transfected with **A** FLAG-tagged β2-integrin or **B** FLAG-tagged β1-integrin for 48 h. Cell lysates were immunoprecipitated with either anti-FLAG mAb and blotted for HA or with anti-HA mAb and blotted for FLAG. **C** Comparison of trans-well migration between CRISPR-control, CRISPR-MOSPD2 clones (M1, M2), and CRISPR-β2-integrin clones (I1, I2) THP- 1 cells toward SDF- 1 and MCP- 1 (100 ng/ml). Cell count is presented. Samples were run in triplicate. Mean ±S.D. is shown. **D** Adhesion of CRISPR-control and β2-integrin-silenced clone I2 THP- 1 cells to a plate coated with different ECM ligands. Relative adhesion based on OD to CRISPR-control is presented. Samples were run in triplicate. Mean ±S.D. is shown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

with UC. CD patients were aged 25–70 (median 38) and had mildly active disease (CDAI 150–220) or moderate disease (CDAI 221–450). At the time of sampling, CD patients were either untreated (IBD- 1) or undergoing treatment with anti-TNF mAb (4/7—57%), anti-integrin α4β7 mAb (vedolizumab), or azathioprine. UC patients were aged 22–66 (median 56), with mild (clinical Mayo score 2–4) or moderate (IBD- 10; clinical Mayo score 5–6) disease. Patients were being medicated at the time of sampling primarily with vedolizumab alone (4/5—80%) or together with mesalazine. One UC patient (IBD- 10) was being treated with anti-TNF mAb. Table 6 shows the clinical characteristics of the CD and UC patients, as well as the efficacy of IW- 601 in blocking ex vivo migration of their peripheral blood monocytes.

Collectively, the results indicate that IW- 601 profoundly inhibits the migration of monocytes isolated from all tested subjects, regardless of disease severity, gender, or the patient's active therapy. Further analysis to determine whether there were significant differences in monocyte migration in response to treatment with IW- 601 between sub-groups of patients (i.e., RRMS vs PPMS; CD vs UC) or between diseases (i.e., MS, RA, PsA, and IBD) came back negative (Fig. 5 and Supp. Table 2).

### Anti-MOSPD2 mAb inhibits disease progression in RA and colitis animal models

We previously demonstrated that treatment with anti-MOSPD2 mAb significantly reduced disease severity in the EAE model of MS [7]. Next to the effect seen with IW-601 on monocyte migration isolated from RA and colitis patients, we explored how treatment with anti-MOSPD2 mAb will affect disease progression in corresponding mouse models. The results depicted in Fig. 6A–B show that treatment with anti-MOSPD2 mAb arrests disease progression in the CIA model and is superior to the treatment with anti-TNF-α mAb, which is frequently used to treat RA patients. In addition, anti-MOSPD2 mAb ameliorated disease severity in the TNBS-induced model of colitis, concurrent with a reduction in the production level of pro-inflammatory cytokines (Fig. 6C–D).

## Discussion

Monocyte recruitment into inflamed tissues involves the interaction of integrins on circulating monocytes with the vascular endothelium and the ECM to enable adhesion and migration. Understanding the mechanisms that control the dynamic changes in integrin conformation on monocytes supports the development of novel treatments for inflammatory diseases. In this study, we show for the first time that MOSPD2 binds to the integrin β2-subunit and controls the conformational state of αLβ2 integrin in monocytes. Although the spatiotemporal dynamics of MOSPD2 are still under investigation, we propose the following mechanism by which MOSPD2 regulates monocyte adhesion and migration (Fig. 7). In the inactive state, β2

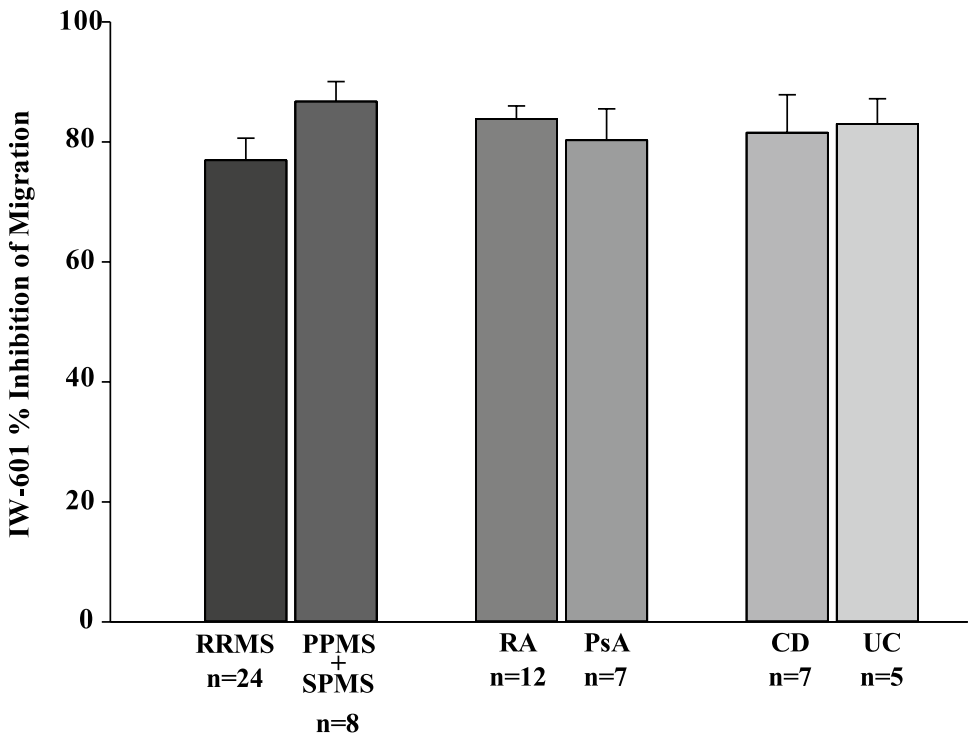



**Fig. 5** Inhibition of monocyte migration by IW- 601. Results are of mean + SEM normalized to IgG control antibody and shown in %. No statistically significant differences between disease types for IW- 601 were observed (one-way ANOVA)

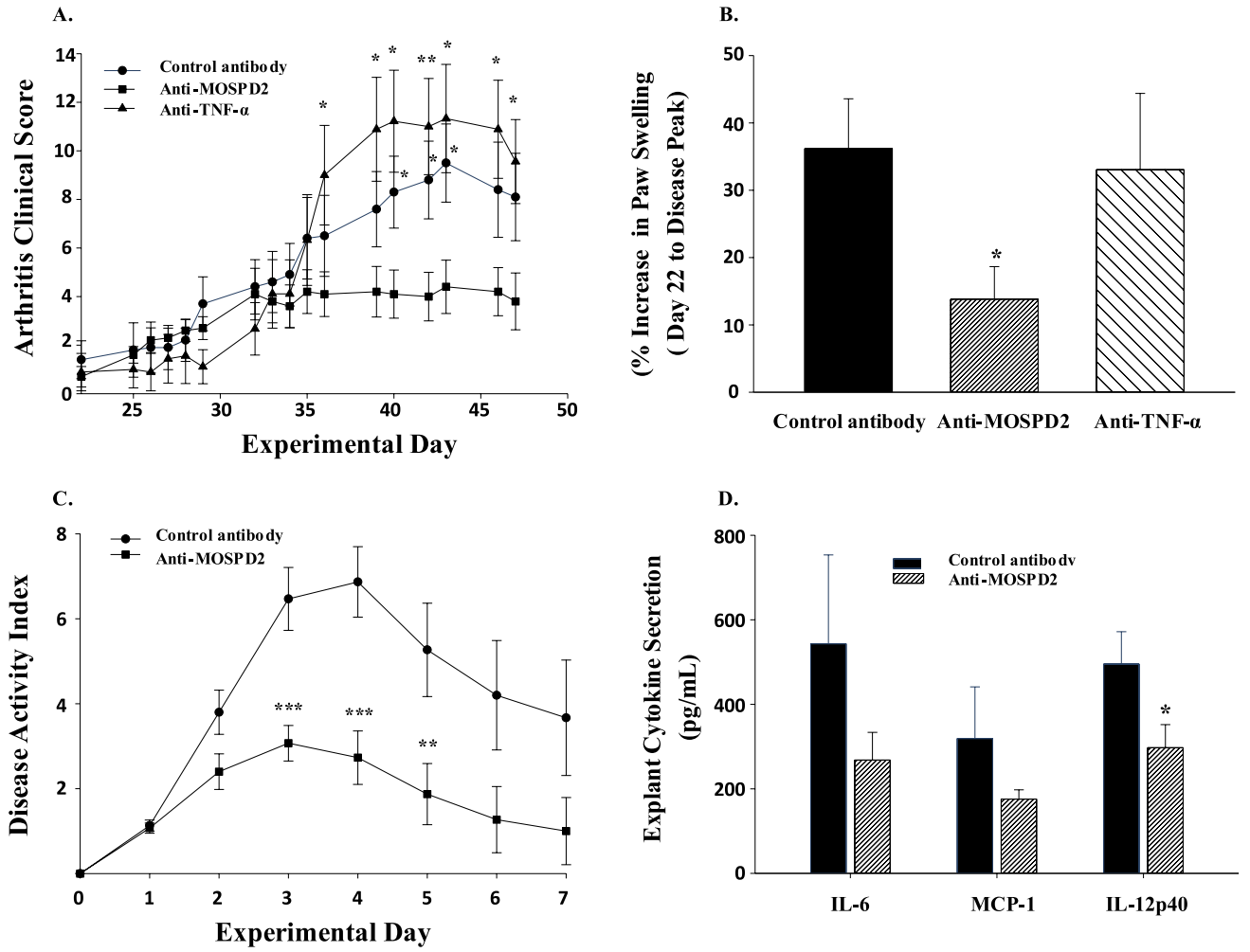
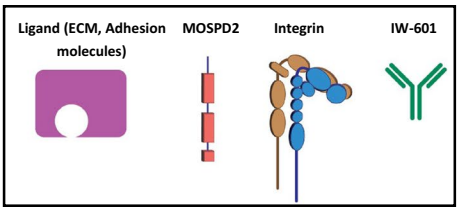
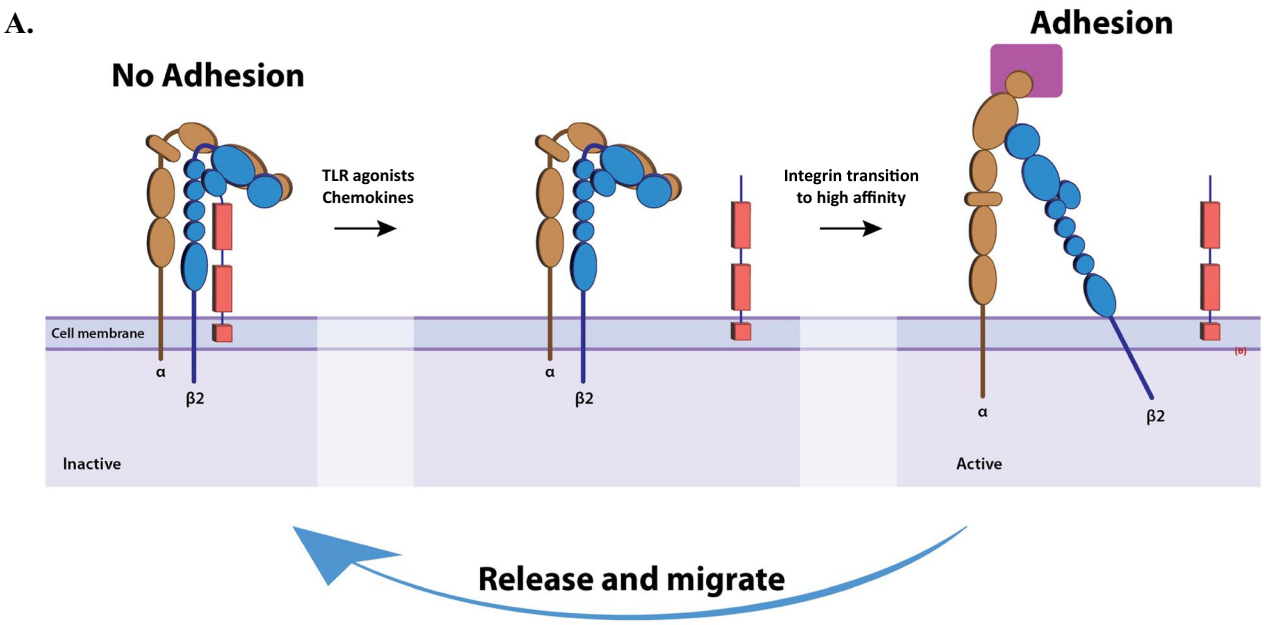



**Fig. 6** Treatment with Anti-MOSPD2 mAb curtails inflammatory disease progression. **A**–**B** Mice were injected with C-II to induce arthritis. On the first day of clinical manifestation (day 22) mice were apportioned ($n = 9$–10/group) to receive isotype control, anti-MOSPD2 mAb, or anti-TNF-α mAb every 3–4 days. Data is presented as mean ± SE. **C**–**D** Colitis was induced in mice ($n = 15$/group) by intra-rectal administration of TNBS. Isotype control or anti-MOSPD2 mAb was injected 2 days before disease induction, on the day of induction (day 0) and 3 days later. Colons for cytokine production were collected upon sacrifice (day 7). Data is presented as mean ± SE. $p$ values were calculated by ANOVA with multiple comparisons test. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$

integrin heterodimers are attached to MOSPD2 through the β2-subunit and adopt the bent-close conformation. Extracellular activation drives the detachment of MOSPD2 from the β2-subunit, allowing the integrin to transition to the high-affinity extended-open conformation for ligand binding and adhesion. Reattachment of MOSPD2 to the β2-subunit shifts the equilibrium back to the bent-closed conformation, decreases the strength of adhesiveness, and permits cell migration to proceed (Fig. 7A). The elements that promote MOSPD2 detachment from the β2-subunit are currently unknown, but they might arise from the inside-out signaling. Employing IW- 601 hinders the interaction between MOSPD2 and the β2-subunit, consequently inducing integrin activation and adhesion and essentially arresting monocyte movement (Fig. 7B). The durable high-affinity conformation of αLβ2 integrin observed in MOSPD2-silenced cells and following treatment with IW-601 suggests a central role for MOSPD2 in β2 integrin dynamics. While such a role was demonstrated only for αLβ2 integrin due to the availability of conformation-specific reagents, it is conceivable that other integrins containing the β2-subunit (i.e., αMβ2, αXβ2, and αDβ2) are similarly regulated by MOSPD2, supported by data from Figs. 1 and 2. It is important to note that although lymphocytes and other cells express various integrins, the presence of surface MOSPD2 is restricted to the myeloid lineage, thus providing a specific way to control adhesion and migration in these cells.

There is compelling evidence that infiltration of blood-borne monocytes into target organs can exacerbate


Ligand (ECM, Adhesion molecules)
MOSPD2
Integrin
IW-601


A.
No Adhesion
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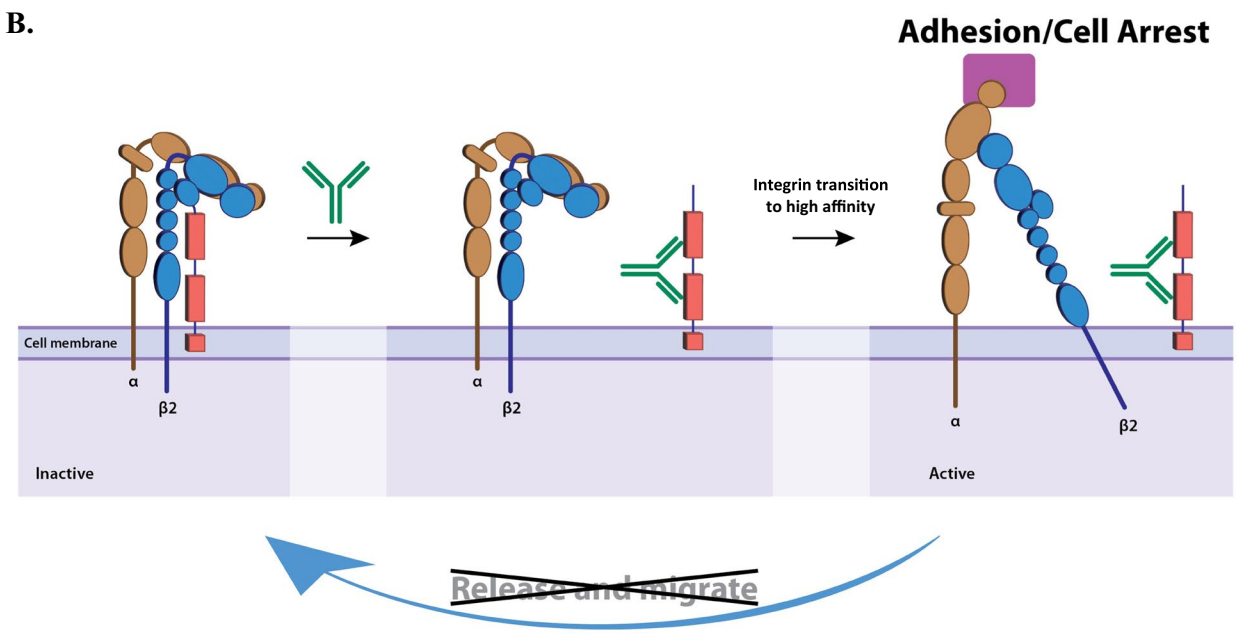
B.
Adhesion/Cell Arrest
Integrin transition
to high affinity
Cell membrane
α
β2
Inactive
Active
Release and migrate

◂**Fig. 7** MOSPD2 is an adhesion checkpoint that regulates the activation state of integrin-β2 on monocytes. **A** MOSPD2 enables the normal β2 integrin conformational changes that are required for monocyte adhesion and migration. **B** Treatment with anti-MOSPD2 mAb IW- 601 keeps β2 integrin in its high-affinity conformation, blocking monocyte ability to detach from the extracellular matrix and migrate into the inflamed tissue

inflammation. Limiting the migration of monocytes from the periphery to inflamed tissues, therefore, has the potential to modulate the pathophysiology of chronic inflammation. We recently reported that anti-MOSPD2 mAbs significantly attenuated CNS inflammation in a mouse model of MS [7]. Based on an understanding of the mechanism by which MOSPD2 governs adhesion and migration, the humanized anti-MOSPD2 mAb IW- 601 was tested here for its potential to inhibit ex vivo migration of monocytes isolated from the peripheral blood of patients diagnosed with various chronic inflammatory diseases. While the number of tested patients varied for each type of disease, we included subjects of both genders, with different levels of disease severity, who were being treated at the time of sampling with diverse medications. The leading drug used for the cohort of RRMS patients was DMF, while others in this group of patients were treated with cladribine, ocrelizumab, natalizumab, teriflunomide, and interferon-beta- 1a, all known to target the number and motility of lymphocytes. Progressive patients were primarily treated with ocrelizumab, which induces B-cell aplasia. Methotrexate, known profoundly to affect T and B cells, prevailed as the leading drug administered to the cohort of RA patients in various combinations, whereas PsA patients were treated mainly with TNF antagonists. Finally, the cohort of colitis patients was treated with vedolizumab, an integrin α4β7 blocker expressed on lymphocytes, complemented with an anti-inflammatory drug (azathioprine/mesalazine), or else, TNF antagonists were applied. Although the samples tested in this study were taken from patients with different chronic inflammatory diseases who were being treated with a broad range of drugs, IW- 601 was able to demonstrate strong ex vivo blocking of monocyte migration. Importantly, that inhibition was independent of disease severity, suggesting that these antibodies could serve as a treatment option for more advanced stages of the disease. The ex vivo human data were further supported by in vivo results in models of IBD and RA, in which treatment with anti-MOSPD2 ameliorated disease severity and arrested disease progression, respectively.

Our findings suggest that anti-MOSPD2 mAbs have therapeutic potential, either as a monotherapy or in combination with current therapies, via a distinct mechanism that arrests innate immune cell movement and prevents their penetration into inflamed tissues.

Taken together, we describe a novel mechanism that uniquely controls monocyte adhesion and migration, and in which MOSPD2 acts as an adhesion checkpoint that regulates monocyte motility by defining the activation state of β2 integrins. This mechanism may be used to develop new therapies for inflammatory diseases in which monocytes are central drivers. Our lead anti-MOSPD2 candidate, IW- 601, is currently being studied in a phase 1 first-in-human clinical trial.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12026-025-09633-6.

**Author contribution** Y.S, P.K, N.Y and O. P-M performed silencing of expression, cloning, adhesion, migration assays, and flow-cytometry analysis. A.K, N.M, O.E and V.F provided human samples and personal data. I.M directed the study and wrote the manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethical approval** All animal experiments described in this paper were approved by the Institutional Animal Care and Use Committee of the Sheba Medical Center, Ramat Gan, Israel. Venous blood samples were drawn from patients in compliance with the Institutional Review Board at the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

**Consent to participate** Signed informed consent was received from all participants prior to sample donation.

**Permission to reproduce material from other resources** Not applicable.

**Competing interests** Niva Yacov, Oshrat Propheta-Meiran, and Itzhak Mendel are employees of ImmuneWalk Therapeutics.



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