Cis interaction between sialylated FcγRIIA and the αI-domain of Mac-1 limits antibody-mediated neutrophil recruitment

Gurpanna Saggu1, Koshu Okubo1, Yunfeng Chen2, Ravi Vattepu3, Naotake Tsuboi1,7, Florencia Rosetti1,8, Xavier Cullere1, Nathaniel Washburn4, Suhail Tahir1, Aaron M. Rosado2, Steven M. Holland5, Robert M. Anthony3, Mehmet Sen6, Cheng Zhu2 & Tanya N. Mayadas1

Vascular-deposited IgG immune complexes promote neutrophil recruitment, but how this process is regulated is still unclear. Here we show that the CD18 integrin Mac-1, in its bent state, interacts with the IgG receptor FcγRIIA in cis to reduce the affinity of FcγRIIA for IgG and inhibit FcγRIIA-mediated neutrophil recruitment under flow. The Mac-1 rs1143679 lupus-risk variant reverses Mac-1 inhibition of FcγRIIA, as does a Mac-1 ligand and a mutation in Mac-1’s ligand binding αI-domain. Sialylated complex glycans on FcγRIIA interact with the αI-domain via divalent cations, and this interaction is required for FcγRIIA inhibition by Mac-1. Human neutrophils deficient in CD18 integrins exhibit augmented FcγRIIA-dependent recruitment to IgG-coated endothelium. In mice, CD18 integrins on neutrophils dampen IgG-mediated neutrophil accumulation in the kidney. In summary, cis interaction between sialylated FcγRIIA and the αI-domain of Mac-1 alters the threshold for IgG-mediated neutrophil recruitment. A disruption of this interaction may increase neutrophil influx in autoimmune diseases.
cyRIIA, a uniquely human ITAM-containing receptor, is a key activating receptor for complexed IgG present on multiple myeloid cells and platelets. For example, FcyRIIA on dendritic cells is essential for generating anti-tumor T cell responses whereas on neutrophils promotes cytotoxic functions and on macrophages facilitates immune complex (IC) clearance. Moreover, studies have identified a key role for FcyRIIA, and other low affinity FcγRs, in neutrophil and monocyte capture by IgG-immune complexes deposited on the endothelium in vitro and in vivo. FcyRIIA also positively regulates Toll-like (TLRs) and cytokine receptors and FcyRIIA SNPs associate with a range of diseases from SLE and rheumatoid arthritis to Kawasaki disease. Thus, determining how FcyRIIA activity is regulated may have broad significance for disease pathogenesis. Here, we studied the mechanisms that regulate FcyRIIA mediated neutrophil recruitment to deposited immune complexes, potentially one of the earliest steps of inflammation and subsequent tissue damage in autoimmune disease.

The CD18 integrins, composed of a common CD18 (β2) subunit complexed with unique CD11 (α) subunits (CD11a–d), are a major family of adhesion molecules on myeloid cells. CD18 integrin binding to ligand relies on allosteric changes transmitted to and from the ligand binding αI-domain, which has a divergent cation in the metal ion dependent adhesion site (MIDAS) that coordinates acidic residues in the ligand. Allostery relay is triggered by inflammatory mediators or heterologous receptors, which generate intracellular signals that impinge on the cytoplasmic tail of the CD18 subunit. These signals shift the integrin to a bent/closed to various degrees of active/open, extended conformations that have increased ligand binding affinity. Historically, CD18 integrins have been considered to be pro-inflammatory. Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18) promote neutrophil recruitment with an absence of all CD18 integrins in Leukocyte adhesion deficiency I (LAD1) patients leading to recurrent infections. Mac-1 also contributes to sustained inflammation and tissue damage and enhances the function of heterologous receptors such as Toll-like receptors (TLRs), FcyRs and the urokinase receptor (uPAR) by regulating intracellular signaling. However, Mac-1 can also inhibit TLR function. A similar duality in Mac-1 function may exist for FcyRs.

Results

To examine Mac-1 effects on FcyRIIA function, we generated stable Jurkat T cell lines, previously mutagenized to lack CD18 integrin LFA-1 (J)34, that express human FcyRIIA in the absence (J-IIA) or presence of human Mac-1 (J-IIA Mac1). We also expressed human FcyRIIA on Jurkat cells with intact endogenous LFA-1 expression (J-IIA LFA1). The ratio of FcyRIIA to Mac-1 and LFA-1 was higher in the J-IIA Mac1 and J-IIA LFA1 than in healthy, normal human and mouse neutrophils, so the impact of Mac-1 and LFA-1 on FcyRIIA may be underestimated in these cells (Supplementary Figure 1A). FcyRIIA expression was similar across all cell lines (Supplementary Figure 1B). We evaluated adhesion of the Jurkat cell lines to plate-immobilized ICs under physiological shear flow conditions. J-IIA but not J cells robustly bound immobilized ICs, which was significantly reduced in J-IIA Mac-1 cells. A comparable decrease was observed in J-IIA LFA1 cells. The reduction in binding of J-IIA Mac-1 and J-IIA LFA1 cells was rescued by treatment of cells with a phorbol ester (PMA), a known potent activator of PKC and CD18 integrins (Fig. 1a), while PMA had no effect on J-IIA binding (Fig. 1a). Moreover, binding of J-IIA Mac-1 was partially rescued when ICs were co-immobilized with the CD18 integrin ligand ICAM-1 (Fig. 1a). Notably, J-IIA Mac-1 cells did not adhere to ICAM-1 alone (Fig. 1a) likely because of the need for IC engagement of FcγR to activate Mac-1 to bind ICAM-1. Notwithstanding the reduction in binding of J-IIA Mac1 cells, the cells that bound exhibited increased spreading on ICs compared to J-IIA cells (Fig. 1b), as expected from previous studies.

Next, we assessed the effect of Mac-1 and LFA-1 on FcyRIIA mediated binding to TNF stimulated human dermal microvascular endothelial cells (HDMEC) coated with anti-endothelial cell antibody (AECA), which models anti-endothelial cell antibody (AECA) deposition observed in autoimmune patients. AECA enhances neutrophil adhesion to the TNF activated endothelium (TNF/AECA) under flow that is FcyRIIA dependent. In the Jurkat cell lines, J-IIA cells were recruited only when ICs were present and this was inhibited by a FcyRIIB blocking antibody. J-IIA Mac1 cells exhibited greater binding to TNF treated endothelial cells compared to J-IIA that is attributed to Mac-1 binding to ICAM-1 on the endothelium. In contrast, J-IIA Mac1 binding to TNF/AECA was significantly reduced compared to J-IIA (Fig. 1c). A similar result was obtained with J-IIA LFA1 (Fig. 1d). On the other hand, FcyRIIA had no effect on Mac-1 and LFA-1 binding to ICAM-1. Interestingly, engineered sialylation with soluble glycosyltransferases in vivo was recently shown to reduce inflammation and tissue injury in models of arthritis and nephroptotic nephritis. Here, we test the hypothesis that an ectodomain cis-interaction between FcyRIIA and bent Mac-1 represents a mechanism for regulating FcyRIIA affinity for IgG and subsequent immune complex-mediated neutrophil recruitment. We show that a divalent cation-dependent cis interaction between the α5-helix of the Mac-1 ligand binding αI-domain, E253-R261, and the heavily sialylated ectodomain of FcyRIIA reduces FcyRIIA affinity for IgG and subsequent neutrophil recruitment to deposited ICs under physiological flow conditions. Using neutrophils from patients with LAD1 and a mouse model of acute anti-GBM nephritis with neutrophil selective changes in CD18 expression, we show that FcγR-mediated neutrophil recruitment is negatively regulated by CD18 integrins. Thus, modulation of FcyRIIA sialylation and Mac-1 allostery may represent a mechanism for fine tuning FcyRIIA responses to IgG immune complexes in myeloid cells.
**Fig. 1** CD18 integrins, Mac-1 and LFA-1 in cis inhibit FcγRIIA mediated interaction with ICs under flow. Cells were perfused under physiological flow conditions over plate immobilized ICs (a, b) or TNF activated human dermal microvascular endothelial cells (HDMEC) with or without ICs generated by incubating cells with mouse anti-endoglin mAb (anti CD105) and rabbit anti-mouse IgG (c, d). The number of adherent cells was assessed and averaged. a Jurkat cells expressing FcγRIIA (J-IIA) with Mac-1 (J-IIA Mac1) or with LFA-1 (J-IIA LFA1) were perfused over immobilized ICs or ICs co-immobilized with ICAM with or without prior PMA treatment. Left panel shows cell adhesion to immobilized ICs at the indicated shear stress. Data is presented as average ± SD of one representative of four experiments with duplicate coverslips per condition. Right panel, bar graphs represent fold change compared to J-IIA cells at 1.0 dyne/cm² under indicated conditions. Data is presented as average ± SEM. b After perfusing cells over immobilized ICs, the coverslips were fixed, permeabilized, and stained with rhodamine-phalloidin to visualize the actin cytoskeleton. Bar graphs represent cell area. Data is presented as average ± SEM of n = 3. c, d Indicated Jurkat cells were perfused over TNFα-activated HDMEC with or without deposited ICs. The number of adherent cells was assessed. No rolling was observed. Cells were pre-treated where indicated with anti-FcγRIIA (IV.3) or isotype controls for 30 min at 37 °C. Bar graphs represent fold change compared to J-IIA TNF/IC. Data is presented as average ± SEM of n = 3. e Isolated human peripheral blood PMNs from 3 normal donors (control) and 3 LAD1 patients were pre-treated where indicated with anti-FcγRIIA (IV.3) and perfused across TNFα activated HDMEC with or without IC coating and analyzed as in c. Results for each of the three LAD1 patients with 15.3, 17.9, and 22.7% CD18 compared to their respective healthy donors (Con) is shown. Data is average fold change compared to TNF alone control ± SD from duplicate coverslips. For a, b *p < 0.05; ***p < 0.001 using the Student’s unpaired t-test. For c-e p < 0.05; ***p < 0.001 using the one way ANOVA followed by Sidak’s Multiple comparison test.
mediated cell adhesion to TNF activated endothelium (Supplementary Figure 1C) suggesting that although Mac-1 inhibits FcyRIIA activity, the converse does not occur. To evaluate the relevance of our findings in human neutrophils, we compared the binding of neutrophils from normal human donors to TNF activated and/or TNF/AECA coated HDMECs with neutrophils from three patients with Leukocyte Adhesion Deficiency 1 (LAD1)\textsuperscript{38}, which had equivalent surface levels of FcγRIIA and FcγRIIB but reduced CD18 integrins compared to normal neutrophils (Supplementary Figure 1D). Notably, although CD11b biosynthesis is not dependent on CD18, an unassociated CD11b has improper topology due to incomplete folding of its β-propeller domain\textsuperscript{39} and is thus most likely to be incompetent for ligand binding. Normal human neutrophils bound to TNF activated HDMEC while LAD1 neutrophils rolled but did not adhere (Fig. 1e) as expected\textsuperscript{38}. Normal human neutrophils exhibited an increase in binding to TNF/AECA endothelial cells, which was inhibited by a functional blocking anti-FcγRIIA antibody (Fig. 1e), as previously reported\textsuperscript{36}. LAD1 neutrophils robustly bound TNF/AECA endothelial cells despite the low to absent binding to endothelium activated with TNF alone and this was completely FcyRIIA dependent (Fig. 1e). The results with LAD1 neutrophils is in seeming contradiction to the reported inhibition of neutrophil binding to TNF/AECA endothelial cells when anti-CD18 integrin antibody TS1/18 is added\textsuperscript{40}. Importantly, this report also showed that ICAM-1, the chemokine IL-8, released by the TNF activated endothelium and its receptor CXCR1/2 on the neutrophil\textsuperscript{3}, which induces integrin activation by inside-out signaling\textsuperscript{40} are also required for neutrophil binding to TNF/AECA endothelial cells. A model depicted in Supplementary Figure 2, reconciles both sets of results: In normal neutrophils flowed across TNF/AECA endothelial cells, IL-8/ CXCR1-mediated integrin activation leads to integrin binding to ICAM-1 in trans, which releases the inhibition of FcγRIIA. TS1/18 antibody prevents integrin extension and trans interaction with ICAM-1 thus resulting in continued inhibition of FcγRIIA. AECA coating on unactivated endothelial cells fails to support neutrophil adhesion\textsuperscript{5,6} due to the absence of IL-8/CXCR1 for integrin activation, low surface ICAM-1 for integrin binding, and an absence of mediators that may upregulate FcγRIIA activity\textsuperscript{41,42}. LAD1 neutrophils fails to bind to ICAM-1 on TNF activated endothelial cells as this requires the CD18 integrins. However, they robustly bind TNF/AECA endothelial cells because CD18 deficiency leads to a release of FcγRIIA for binding to IgG-ICs. CD18 integrin inhibition of FcγRIIA is more dramatic in J-IIA Mac-1 and J-IIA LFA-1 cell lines (Fig. 1d) than in neutrophils as Jurkats cells, which are an immortalized line of human T cells, lack CXCR1/2 and therefore cannot respond to IL-8 to activate integrins in the context of the TNF activated endothelium.

**Mac-1 αl domain lowers FcγRIIA affinity for IgG.** Our analysis of the crystal structures of CD18 integrins\textsuperscript{43,44}, FcγRIIA\textsuperscript{45} and IgG\textsuperscript{46} combined with a report that Mac-1 αl domain can interact with FcγRIIA ectodomain\textsuperscript{37} led us to propose a spatial model wherein Mac-1 in its bent/open conformation interacts with FcγRIIA laterally via its ligand binding αl domain to curtail trans-interaction of FcγRIIA with IgG-ICs (Fig. 2a). Fluorescence lifetime imaging microscopy (FLIM) analysis of J-IIA Mac1 cells, using antibodies to the ligand-binding region of FcγRIIA and to the Mac-1 ligand binding αl domain, suggested a close proximity between these two regions on the cell surface of J-IIA Mac-1 cells and human neutrophils (Fig. 2b). Next, direct molecular interactions were examined using a biomembrane force probe (BFP). We measured the adhesion frequency versus contact time between J-IIA and J-IIA Mac1 cells and beads immobilized with recombinant Mac-1 presented in trans. J-IIA cells interacted with Mac-1 in trans and this interaction was weakened when Mac-1 was present on the cell surface alongside FcγRIIA in cis, as reflected by the significantly lowered effective affinity (A_Kc) (Fig. 2c). Note that this lower A_Kc is an averaged value over all the FcγRIIA copies on the cell surface; this included the ones engaged by cis Mac-1 and therefore adopting low affinity for trans Mac-1 binding, and the others free from cis Mac-1 interaction whose affinity for trans Mac-1 should remain unchanged. Also, using J-Mac1 and J-IIA Mac1 cells and bead-immobilized FcγRIIA we observed that Mac-1 interacted with FcγRIIA in trans and this interaction was reduced when FcγRIIA was present with Mac-1 in cis (Fig. 2d). These data suggest that Mac-1 and FcγRIIA directly interact in cis, which hinders their respective trans interaction to their counterparts on an opposing surface. To examine whether Mac-1 reduces FcγRIIA affinity for ligand, we used J-IIA cells and J-IIA Mac1 cells presented with IgG-coated beads. We found that the A_Kc of J-IIA Mac1 cells binding to the IgG-coated beads was significantly lower compared to J-IIA cells (Fig. 2e). In addition, the IgG off-rate was higher for J-IIA-WT compared to J-IIA cells suggesting that Mac-1 may be able to compete with established FcγRIIA-IgG binding (Fig. 2e). To assess the role of Mac-1’s ligand binding αl domain in the observed inhibition of FcγRIIA we used two approaches. First, we tested the J-IIA Mac1 cells with neutrophil inhibitory factory (NIF), a protein from hookworm that binds to the Mac1 αl domain\textsuperscript{47,48} at E253-R261 49. Second, we generated J-IIA cells expressing Mac-1 mutated in the E253-R261 region of the αl domain that had surface levels of FcγRIIA and Mac-1 that were comparable to J-IIA Mac1 cells (Supplementary Figure 1B). Both J-IIA Mac1 cells treated with NIF and JIIA cells expressing the Mac-1 E253-R261 mutant failed to reduce FcγRIIA affinity for bead immobilized IgG (Fig. 2f). Thus, the αl-domain of Mac-1 interacts with the ectodomain of FcγRIIA through its E253-R261 NIF binding region to reduce the affinity of FcγRIIA for IgG. Accordingly, the proximity of Mac-1 E253-R261 to FcγRIIA was reduced compared to wild-type Mac-1 as assessed by FLIM (Fig. 2b).

Next, we show that the interaction of Mac-1 αl-domain with FcγRIIA has functional consequences. J-IIA cells pretreated with recombinant, soluble αl-domain of Mac-1 exhibited a reduction in binding to immobilized ICs and to TNF/AECA endothelial cells under flow (Fig. 3a). Treatment of J-IIA Mac-1 cells with NIF reversed Mac-1 inhibition of FcγRIIA in both flow assays and the inhibitory effect of Mac-1 was also abolished in the E253-R261 mutant (Fig. 3b). Importantly, the E253-R261 mutant had no effect on FcγRIIA mediated cell spreading on ICs (Fig. 3c). In contrast to the inhibitory role of Mac-1 on FcγRIIA, Mac-1 had no effect on human FcγRIIB interaction with ICs as assessed in flow assays using Jurkat cell lines engineered to stably express human FcγRIIB in the absence (J-IIIB) or presence (J-IIIB Mac1) of Mac-1 (Fig. 3d). As Mac-1 has been shown to inhibit TLR4 function\textsuperscript{19,50}, we also tested whether Mac-1 interacts with TLR4 in cis. FLIM analysis revealed that these two receptors were not in close proximity on the surface of human neutrophils (Supplementary Figure 3).

In summary, the interaction of E253-R261 in the αl-domain of Mac-1 with FcγRIIA’s ectodomain curtails FcγRIIA’s affinity for ICs, and therefore cell capture under flow, an inhibition that is reversed by Mac-1 activation and Mac-1 ligand (NIF) binding.
reduces 2D ligand-binding affinity and force-regulated dissociation of receptor-ligand bonds by perturbing allostery relay from the β-propeller in which it resides to the ligand binding α-domain. The R77H phenotype could be rescued by forcing the tail and lower leg separation of the α and β subunits in the absence of constitutive integrin extension and headpiece opening. Given these data, we considered the possibility that the interaction of Mac-1 with FcRIIA, which we propose serves as a “cis ligand”, will be similarly perturbed by R77H due to defects in allostery relay to the αl-domain of Mac-1. To address this hypothesis, we generated a stable Jurkat cell line expressing the αl-domain of the R77H variant of Mac-1 and FcRIIA with surface levels of FcRIIA and Mac-1 similar to J-IIA Mac-1 cells (Supplementary Fig. 1B). FLIM analysis revealed that the αl-domain of the R77H variant of Mac-1 did not remain in close proximity to FcRIIA compared to WT Mac-1 (Fig. 4a). In addition, in a BFP assay that
Mac-1 αl-domain interacts with sialylated FcRIIA. Given the importance of Mac-1 αl domain in binding FcRIIA and the reports of FcRIIA glycosylation we posited that the divalent cation in the MIDAS motif of the αl-domain, known to be essential for Mac-1 ligand binding, interacts with potential negatively charged sialic acids on FcRIIA to reduce FcRIIA binding affinity for ICs as depicted in Fig. 5a. FcγRIIA has been shown to contain two consensus N-glycosylation motifs, N64 and N145 located in the first and second Ig-like extracellular domains, respectively. These two N-glycans are distant from the FcγR:Fc interface and neither participates directly in the interaction based on published co-crystal structures. Characterization of site-specific glycosylation for FcγRIIA on neutrophils showed that the two sites have distinct glycosylation patterns. Glycosylation at N64 is characterized by sialylated bi- and tri-antennary complex type glycans. The glycans at N64 were almost entirely sialylated, with >95% targeted. In contrast, the glycosylation at N145 was characterized by a mixture of non-sialylated high mannose type, sialylated hybrid type and sialylated fucosylated complex type glycans. The glycans at N64 are core fucosylated complex type N-glycans with up to 3 antennae and up to two sialic acid residues (Fig. 5b) (Supplementary Table 1). The sialylated structures were detected for this site suggesting full site occupancy. In contrast, the glycosylation at N145 was characterized by a mixture of non-sialylated high mannose type, sialylated hybrid type and sialylated biantennary complex type glycans. The glycans at N64 were almost entirely sialylated, with >95% targeted. In contrast, the glycosylation at N145 was characterized by a mixture of non-sialylated high mannose type, sialylated hybrid type and sialylated biantennary complex type glycans. The glycans at N64 are core fucosylated complex type N-glycans with up to 3 antennae and up to two sialic acid residues (Fig. 5b) (Supplementary Table 1). The sialylated structures were detected for this site suggesting full site occupancy.

Mac-1 αl-domain reduces FcγRIIA binding to ICs, but does not affect cell spreading or FcγRIIB binding to ICs. Jurkat cells expressing either FcγRIIA or FcγRIIB and Mac-1 with or without pretreatment with recombinant NIF (10 nM) (b), or J-IIA cells expressing Mac-1-E253,R261 mutant (b) were perfused at 1 dyne/cm² over IC-coated coverslips (a and b, left panels) or TNF activated and IC coated HDMEC on coverslips (a and b, right panels). The number of adherent Jurkats was assessed and averaged. Data are represented as fold change compared to J-IIA cells of an average of 3 experiments ± SEM. **p < 0.01, ***p < 0.001 using the One way ANOVA followed by Sidak’s Multiple comparison test.
capped with a single N-acetylneuraminic acid linked either α2–6 or α2–3 to the penultimate galactose. The relative abundance of sialylated glycans ranged from 30–60% across the 5 donors. The non-sialylated N-glycans were high mannose glycans ranging from 5–9 mannose residues. The relative abundance of these species ranged from 40–70% across the 5 donors. This site also appears to be fully occupied based on the absence of the aglycosylated peptide. Mass spectrometry was also used to compare the glycosylation of the conserved N64 and N65 site in FcγRIIA and FcγRIIIB, respectively that was isolated from transfected...
Jurkat cells, JIA and JIIB. The N64 from FcyRIIA was highly sialylated (Fig. 5c) while those at N145 were a mixture of high mannosae, hybrid and complex sialylated, as observed for FcyRIIA in human neutrophils (Fig. 5b). In contrast, the N65 site of FcyRIIB was predominantly (>90%) unoccupied (Fig. 5c), consistent with observations in human neutrophils (Washburn et al., Manuscript in preparation). The discrepancy in occupancy of N64/N65 between the two proteins may be explained by differences in the area around these residues in the 3D protein structure. One major difference is the presence of an N-glycan at N45 of FcyRIIB that is not present in FcyRIIA. Based on the crystal structures for the two proteins this glycan could significantly influence the accessibility of N65 to glycosyltransferases. Indeed, a recent study of the highly homologous FcyRIIIA using NMR and computer simulation revealed an unexpected contact between the glycan at N45 and the protein backbone around N6552. The lack of sialylation of N65 in FcyRIIB, may explain the selective inhibitory effect of Mac-1 on FcyRIIA (Fig. 3a, b) but not FcyRIIB (Fig. 3d).

To further define the molecular interaction between FcyRIIA and Mac-1 αI-domain, we evaluated binding of recombinant GST-αI-domain with FcyRIIA using a solid phase assay. Soluble, recombinant FcyRIIA bound to GST-αI-domain coated plates significantly more than to GST and the former was inhibited by pre-treatment of the GST-αI-domain with NIF (Fig. 5d), showing specificity of the interaction. EDTA treatment significantly reduced FcyRIIA binding to GST-αI-domain suggesting a requirement for divalent cations in the observed interaction (Fig. 5d). Furthermore, neuraminidase treatment of FcyRIIA, which removed all sialic acid, markedly decreased FcyRIIA-αI-domain interactions suggesting a role for sialylation of FcyRIIA in FcyRIIA-αI-domain interactions (Fig. 5d). Next, we determined whether sialylation of FcyRIIA was required for Mac-1 inhibition of FcyRIIA mediated interactions with ICs under flow. Neuraminidase treatment of J-IIA Macl cells reversed Mac-1 inhibition of FcyRIIA while having no effect on J-IIA binding (Fig. 5e). Neuraminidase treatment did not affect J-IIA Mac1 mediated spreading on ICs (Fig. 5f). Similarly, human neutrophil binding to TNF/AECA coated HDMECs under flow was enhanced by neuraminidase pre-treatment (Fig. 5g), while it had no effect on neutrophil binding to endothelial cells treated with TNF alone (Fig. 5g). Together, these data demonstrate that FcyRIIA-αI-domain interactions require sialylation of FcyRIIA and divalent cations and that asialylation of FcyRIIA or removal of divalent cations interferes with Mac-1 inhibition of FcyRIIA and subsequent binding of cells to ICs under low.

Discussion

The dynamic regulation of immune receptors is critical for spatially and temporally tailoring the immune response. We demonstrate that the activity of FcyRIIA, a key activating FcγR on myeloid cells, is inhibited via its cis-ectodomain interaction with the integrin Mac-1. The inhibition is evident when rapid, high affinity interactions between FcyRIIA and IgG are required as in the case of neutrophil capture by ICs under shear stress conditions. However, once productive FcyR-IgG contacts are established, Mac-1 cooperates with FcyRIIA to promote cell spreading, a well-documented role for this integrin19,23. The downregulatory mechanism described herein may be particularly important in neutrophils as the inhibitory FcyRIIB (CD32b), which sets the threshold for activation of innate immune effector cells and B cells31 is low to absent on neutrophils of most individuals32. The observed interaction between Mac-1 ligand binding αI-domain and FcyRIIA on the cell surface, and the dependence of this on divalent cations suggests that FcyRIIA is a cis-ligand of Mac-1. This is consistent with a previous study that reported a cis interaction between FcyRIIA and Mac-1 αI-domain37. In that case, the cis interaction was shown to promote cell migration albeit this only occurred when the FcyRIIA cytoplasmic tail was deleted37. These findings suggest the possibility that Mac-1 may exclude FcyRIIA from “signaling rich” lipid rafts, where FcyRIIA has been shown to accumulate in the absence of its cytoplasmic tail39, and thus reduce FcyRIIA intracellular signaling required for rapid FcyRIIA binding to complexed IgG69. This concept is supported by a recent finding that proteins affixed to the cortical actin cytoskeleton may serve as “pickets” that restrict FcyR lateral
Thus, Mac-1 could serve as a "picket", a function that is overcome when FcγR is cross-linked by ligand and/or when Mac-1 is activated. Notably, TLR4 did not interact with the αI-domain of Mac-1 in cis suggesting that the ectodomain mechanism of FcγRIIA inhibition by Mac-1 does not apply for all receptors known to cooperate with Mac-1. The αI-domain of a bent integrin interacts with FcγRIIA, a cis ligand, as assessed by molecular modeling and FLIM, which is consistent with the emerging concept that a CD18 integrin in its bent conformation can accommodate ligand binding\(^{43}\). The lupus risk SNP, R77H is
productive binding, as we have described for Mac-1 binding to its variant. Average relative abundance for n = 5 donors are shown for both sites in the context of the crystal structure of the FcRIIA-R131 variant. Site-specific glycopeptide analysis of FcRIIA-R131 variant and FcRIIB NA2 allele expressed in Jurkat cells. Plates coated with GST-α domain or GST were incubated with FcRIIA or neuraminidase A treated FcRIIA (asialylated). As indicated, EDTA was included in the buffer to chelate divalent cations. For experimental controls, GST was incubated with FcRIIA and GST-α domain was incubated with NIF to show α-domain specificity. Lectin blots were performed to detect terminal α2,6 sialic acid on FcRIIA in the absence (−) and presence (+) of neuraminidase (Neur). J-ILA and J-Ila Mac1 cells were incubated with neuraminidase for 30 min at 37°C. The cells were diluted 100-fold in flow buffer and drawn over IC-coated coverslips or TNF-stimulated anti-endoglin (IC) coated HDMEC coverslips at 1.0 dynes/cm² and the number of adherent cells were determined as in Fig. 1. Data is presented as fold change compared to J-ILA cells (Neur) of an average of 3 experiments ± SEM. **p < 0.01 using Student’s unpaired t-test. Indicated Jurkat cells were incubated with neuraminidase as in e and seeded onto immobilized ICs for 30 min. Coverslips were fixed, permeabilized, and stained with Alexa 568 phalloidin to visualize the actin cytoskeleton. Bar graphs represent average adherent cell area. Human neutrophils ± neuraminidase (Neur) were drawn across TNF-stimulated HDMEC coated with or without anti-endoglin (IC) at 1 dynes/cm² and the number of adherent cells were evaluated. Data is average of 3 experiments ± SEM. Student’s unpaired t-test. For c–e, **p < 0.05; ***p < 0.001 using the One way ANOVA followed by Sidak’s Multiple comparison test.

Fig. 5 Mac-1 α domain interaction with FcRIIA requires FcRIIA sialylation and divalent cations. a A model of glycosylated FcRIIA and bent Mac-1 with high rotational ability and flexibility of the α domain is depicted. Spheres are Mg2+ (green) and Ca2+ (magenta). The electrostatic surface (−5 [red] to +5 [blue] kT/e) was calculated using the Adaptive Poisson-Boltzmann Solver software plug-in for PyMOL. b Site-specific glycopeptide analysis of human neutrophil derived FcRIIA. Average relative abundance for n = 5 donors are shown for both sites in the context of the crystal structure of the FcRIIA-R131 variant. c Site-specific glycopeptide analysis of FcRIIA-R131 variant and FcRIIB NA2 allele expressed in Jurkat cells. d Plates coated with GST-α domain or GST were incubated with FcRIIA or neuraminidase A treated FcRIIA (asialylated). As indicated, EDTA was included in the buffer to chelate divalent cations. For experimental controls, GST was incubated with FcRIIA and GST-α domain was incubated with NIF to show α-domain specificity. Lectin blots were performed to detect terminal α2,6 sialic acid on FcRIIA in the absence (−) and presence (+) of neuraminidase (Neur). e J-ILA and J-Ila Mac1 cells were incubated with neuraminidase for 30 min at 37°C. The cells were diluted 100-fold in flow buffer and drawn over IC-coated coverslips or TNF-stimulated anti-endoglin (IC) coated HDMEC coverslips at 1.0 dynes/cm² and the number of adherent cells were determined as in Fig. 1. Data is presented as fold change compared to J-ILA cells (Neur) of an average of 3 experiments ± SEM. **p < 0.01 using Student’s unpaired t-test. f Indicated Jurkat cells were incubated ± neuraminidase as in e and seeded onto immobilized ICs for 30 min. Coverslips were fixed, permeabilized, and stained with Alexa 568 phalloidin to visualize the actin cytoskeleton. Bar graphs represent average adherent cell area. h Human neutrophils ± neuraminidase (Neur) were drawn across TNF-stimulated HDMEC coated with or without anti-endoglin (IC) at 1 dynes/cm² and the number of adherent cells were evaluated. Data is average of 3 experiments ± SEM. Student’s unpaired t-test. For c–e, **p < 0.05; ***p < 0.001 using the One way ANOVA followed by Sidak’s Multiple comparison test.
In conclusion, our study revealed an ectodomain interaction between two major adhesion receptors, FcyRIIA and Mac-1 that modulates neutrophil recruitment to deposited IgG, thus providing a mechanism by which the activity of FcyRIIA is regulated in autoimmune mediated neutrophil recruitment. This method of regulation by Mac-1 differs from the intracellular mechanisms described for its regulation of heterologous receptors. Our data also reveals that the interaction of sialylated FcγRIIA with Mac-1 down-regulates FcyRIIA activity, which suggests that cell-type specific sialylation of FcyRIIA on myeloid cells may contribute to diversity in FcyRIIA mediated responses. The calibration of FcyRIIA mediated neutrophil responses to vascular deposited IgCs facilitated by cis interactions with Mac-1, leads us to predict that a similar interaction may affect other FcyRIIA functions that require short duration, high affinity interactions with complexed IgG in myeloid cells expressing these two opsonic receptors (e.g. capture and phagocytosis of live microbes). Finally, our studies suggest that therapeutic agents that maintain Mac-1 allosterically in a bent state may be able to control the deleterious effects of FcyRIIA mediated neutrophil accumulation in autoimmune disease.

**Methods**

**Mice.** Wild type mice (IgGb2/+) and mice lacking all CD18 integrins (IgGb2−/−) were used for immunostaining and measured exactly the same on the microscope. FcyRIIA and Mac-1 were also measured exactly the same on the microscope.

**Reagents and antibodies.** Human IgM (Jackson ImmunoResearch), Protein A (Pierce Thermo Scientific), Human ICAM-1 Fc (R&D), mouse IgG2a clone M1/70 (Biolegend; cat#101216), mouse IgG2a clone 4G7 (eBioscience; cat#14-4321-82), anti-human CD32a clone IV.3 (Stemcell Technologies), Protein A (Pierce Thermo Scientific) as per manufacturer instructions.

**Human neutrophils.** Human polymorphonuclear neutrophils (PMNs; 85% pure) were isolated from whole blood drawn from healthy volunteers. Blood was collected in ACD anticoagulant in BD vacutainer tubes and centrifuged on a histopaque (Sigma) separation medium followed by dextran (1%) sedimentation of RBCs. The isolated cells were washed 8°C and used immediately. De-identified LAD1 patient blood samples drawn at National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Infectious Diseases, National Institutes of Health and age matched normal, healthy controls, were collected in ACED anticoagulant in BD vacutainer tubes and shipped on dry ice. The procedure was repeated a total of five times. Blood from the naive state was collected in BD vacutainer tubes at 37°C. Cells were washed and stained with Alexa 568 phallolidin to visualize the actin cytoskeleton. Adherent cell area was calculated on ImageJ (NIH).

**Biomembrane force probe assays.** Human red blood cells (RBCs) were isolated from whole blood of healthy volunteers by finger prick according to protocols approved by the Institutional Review Board of Georgia Institute of Technology. Freshly isolated human RBCs were biotinylated and glass beads were coated with streptavidin (SA) plus FlhG1, Mac-1, or FcyRIIA as previously described. A coated bead was attached to the apex of a micropipette-aspirated bionitogenated RBC, which together acted as an ultrasensitive force transducer. The aspiration pressure was set to control the probe stiffness at 0.2 or 0.3 pN/nm. The axial displacement of the probe-RBC interface was monitored by high-speed camera, which reflected the force on the RBC by Hooke’s law. Jurkat cells expressing FcyRIIA and/or Mac-1 were aspirated by an opposing micropipette. The Jurkat cell was programmed to repeatedly approach and contact the probe bead to define contact times (t), 0.1–5 s to allow bond formation, which was signified by a tensile force on the RBC upon retraction of the cell. The approach–retraction cycle was repeated 50 times for at least 3 cell–bead pairs at each t to measure the mean SEM of adhesion frequency, P. Then the 2-dimensional (2D) effective affinity (A,K) and off-rate (k_d) were derived by fitting the P vs t to a 2D kinetics model with the following equation:

\[
P_f = 1 - \exp\left(-m_k m_a K_d A_K [1 - \exp(-k_d t)]\right)
\]

where m_k and m_a are the respective site densities of receptor (FcyRIIA or Mac-1) and ligand (hlgG1, Mac-1, or FcyRIIA).

**Lentiviral constructs and Jurkat cells.** The R77 mutation was introduced into the human CD11b using standard PCR approaches. The E253-CD11b was introduced by replacing CD11b’s E253−R261 EDVIFPEADR with SGNIDAAKD present in CD11a49 using an overlap PCR strategy with the following primers: sense 5′-AGGCGCCAATCGATGCTGCAAAAGAGGGAGGAGCTCAGGAAACGC-3′ and antisense 5′-GTCTTGGATCAATGGTTCTGGCGCCGATATCCAAAGGATGCC- C-3′ and the mutation was confirmed by DNA sequencing. Replacement of the binding E253−CD11b domain of LFA-1 is likely disruptive because this region, which corresponds to the α7-helix, is not equal in length in Mac-1 and LFA-1. Mac-1 domain has an additional five residues, which structurally adopts an extra 2.5 helical turn. Replacing this region with the CD11a sequence likely causes a structural change not only in this region but also to peripheral segments on the α-domain that impacts Mac-1’s affinity for FcyRIIA. DNA constructs for CD11bWT, CD11bR261, CD11bE253, FcyRIIA (CD32a), or FcyRIIB (CD16b) were cloned in the lentiviral packaging plasmid pWPI (modified from the Addgene, plasmid #12545, from Didier Trono laboratory, by removing the EGFP cassette). Further a lentiviral construct was transfected with the lentiviral construct (pWPI) with the addition of plasmid pBFP-2x (Addgene, plasmid #12260 and #12259). The laboratory of Didier Trono using lipofectamine (Invitrogen). Supernatant of transfected cells were passed through a 0.45 μm filter and used to transduce wild-type Jurkat cells or Jurkat cells lacking CD11b. All cell lines were sterility sorted on a BD FACS Aria to obtain populations of cells with similar levels of desired proteins on their surface. Clones were cultured in RPMI 1640 supplemented with 10% FCS, 2 mCi of l-glutamine and penicillin/streptomycin (0.1 mg/ml) (Lonza) and used in functional assays.
Vectashield (Invitrogen). Protein interactions were defined by time-correlated single-photon counting FLIM as previously described

**Solid phase binding assay.** 96 well Nunc plates (Thermofisher Scientific) were coated with GST al-domain (20 µg/mL) or GST, followed by blocking with 2% BSA solution for 1 h. After washing with TBST, the buffer plate was incubated for 3 h at room temperature in the presence of FcRIIA (40 µg/mL) or asialylated FcRIIA (40 µg/mL), NIF (20 nM) followed by FcRIIA in TBS-Tween and FcRIIA with 2 mM EDTA. After each step, the plate was washed with TBST and bound FcRIIA was detected using mouse anti-human CD32 antibody (clone IV.5, Stemcell Technologies). Recombinant FcRIIA followed by blocked antibodies was developed with 3,3,5,5-tetramethylbenzidine (TMB) HRP substrate, the reactions were quenched with 2 M sulfuric acid and absorbance was measured at 450 nm.

**Characterization of human FcγRI and FcγRIIb glycosylation.** Neutrophil FcRIIA was isolated from ~5 million neutrophils from blood drawn from 5 healthy donors as part of the Momenta Pharmaceuticals blood donor program. The immunoprecipitated proteins were digested with chymotrypsin for 4 h at room temperature in the presence of FcRIIA (40 µg/mL) or asialylated FcRIIA (40 µg/mL), NIF (20 nM) followed by FcRIIA in TBS and FcRIIA with 2 mM EDTA. After each step, the plate was washed with TBST and bound FcRIIA was detected using mouse anti-human CD32 antibody (clone IV.5, Stemcell Technologies). Recombinant FcRIIA followed by blocked antibodies was developed with 3,3,5,5-tetramethylbenzidine (TMB) HRP substrate, the reactions were quenched with 2 M sulfuric acid and absorbance was measured at 450 nm.

**References**

1. Bournozos, S. & Ravetch, J. V. Fcγrgamma receptor function and the design of vaccination strategies. Blood 103, 224–233 (2004).

2. Gillis, C., Gouel-Cheron, A., Jonsson, F. & Bruhns, P. Contribution of human FcgammaRIs to disease with evidence from human polymorphisms and transgenic animal studies. Front. Immunol. 5, 254 (2014).

3. Coxon, A. et al. Fc gamma RIIIA mediates neutrophil recruitment to immune complexes. A mechanism for neutrophil accumulation in immune-mediated inflammation. Immunity 14, 693–704 (2001).

4. Tsuoi, N., Asano, K., Lauterbach, M. & Mayadas, T. N. Human neutrophil Fc gamma receptor initiates and plays specialized nondrug roles in antibody-mediated inflammatory diseases. Immunity 28, 833–846 (2008).

5. Florey, O. J., Johns, M., Esho, O. O., Mason, J. C. & Haskard, D. O. Antiendothelial cell antibodies mediate enhanced leukocyte adhesion to cytokine-activated endothelial cells through a novel mechanism requiring cooperation between Fg(amma)RIa and CXC(3,4)R1. Blood 109, 3881–3889 (2007).

6. Nishi, H. et al. Neutrophil FcγRIIA promotes IgG-mediated granulocyte-macrophage colony-stimulating factor via Abl/Src kinases. J. Clin. Invest. 127, 3810–3826 (2012).

7. Olaru, F. et al. Intracapillary immune complexes recruit and activate slan-expressing CD16+ monocytes in human lupus nephritis. JCI Insight 3, 96492 (2018).

8. Valenzuela, N. M., Trinh, K. R., Mulder, A., Morrison, S. L. & Reed, E. F. Monocyte recruitment by HLA IgG-activated endothelium: the relationship between IgG subclass and FcgammaRIIa polymorphisms. Am. J. Transplant. 15, 1505–1518 (2015).

9. Dunn-Sigrist, I. et al. Pivotal involvement of Fcgamma receptor IIIa in the neutralization of lipopolysaccharide signaling via a potent novel anti-TLR4 monoclonal antibody 15C1. J. Biol. Chem. 282, 34817–34827 (2007).

10. Iwashiv, L. B. Cross-regulation of signaling by ITAM-associated receptors. Nat. Immunol. 10, 340–347 (2009).

11. Duits, A. J. et al. Skewed distribution of IgG Fc receptor IIa (CD132) polymorphism is associated with renal disease in systemic lupus erythematosus patients. Arthritis Rheum. 38, 1832–1836 (1995).

12. Radstake, T. R. et al. Role of Fcgamma receptors IIa, IIia, and IIib in susceptibility to rheumatoid arthritis. J. Rheumatol. 30, 928–933 (2003).

13. Khok, C. C. et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat. Genet. 43, 1241–1246 (2011).

14. Shresha, S. et al. Role of activating FcgammaR gene polymorphisms in Kawasaki disease susceptibility and intravenous immunoglobulin response. Circ. Cardiovasc. Genet. 5, 309–316 (2012).

15. Williams, M. R., Aucutt, V., Newton, G., Alcaide, P. & Luscminskas, F. W. Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol. 32, 461–469 (2011).

16. Arnaout, M. A. Biology and structure of leukocyte beta2 integrins and their role in inflammation. F1000Research 5, 1–13 (2016).

17. Luo, B. H., Carman, C. V. & Springer, T. A. Structural basis of integrin regulation and signaling. Annu. Rev. Immunol. 25, 619–647 (2007).

18. Harris, E. S., Weyich, A. S. & Zimmerman, G. A. Lessons from rare maladies: leukocyte adhesion deficiency syndromes. Curr. Opin. Hematol. 20, 16–25 (2013).
19. Rosetti, F. & Mayadas, T. N. The many faces of Mac-1 in autoimmune disease. *Immunol. Rev.* **269**, 175–193 (2016).
20. Wang, L. et al. Indirect inhibition of Toll-like receptor and type I interferon responses by ITAM-coupled receptors and integrins. *Immunity* **32**, 518–530 (2010).
21. Reed, J. H. et al. Complement receptor 3 influences toll-like receptor 7/8-dependent inflammation: implications for autoimmune diseases characterized by antibody reactivity to ribonucleoproteins. *J. Biol. Chem.* **288**, 9077–9083 (2013).
22. Yee, N. K. & Hamerman, J. A. beta(2) integrins inhibit TLR responses by interfering with the recruitment of TLR7 (CD74) to lipid rafts. *J. Immunol.* **187**, 3208–3217 (2011).
23. Harris, L. J., Larson, S. B., Hasel, K. W. & McPherson, A. Refined structure of an intact IgG2a monoclonal antibody. *Biochemistry* **36**, 1581–1597 (1997).
24. Sen, M. & Springer, T. A. Leukocyte integrin alphaLbeta2 headpiece structures: The alpha domain, the pocket for the internal ligand, and concerted movements of its loops. *Proc. Natl Acad. Sci. USA* **113**, 2940–2945 (2016).
25. Ramsland, P. A. et al. Structural basis for Fc gammaRIIa recognition of human IgG and formation of immunological signaling complexes. *J. Immunol.* **187**, 3208–3217 (2011).
26. Muchowski, P. J. et al. Functional interaction between the integrin antagonist neutrophil inhibitory factor and the I domain of CD11b/CD18. *J. Biol. Chem.* **269**, 26419–26423 (1994).
27. Zhang, L. & Plow, E. F. Identification and reconstruction of the binding site within alphaMbeta2a for a specific and high affinity ligand, NIF. *J. Biol. Chem.* **272**, 17558–17564 (1997).
28. Rosetti, F. et al. Human lupus serum induces neutrophil-mediated organ damage in mice that is enabled by Mac-1 deficiency. *J. Exp. Med.* **200**, 197–222 (2000).
29. Tang, T. et al. Role for Mac-1 (CD11b/CD18) in immune complex-stimulated neutrophil function in vivo: Mac-1 deficiency abrogates sustained Fcgamma receptor-dependent neutrophil adhesion and complement-dependent proteinuria in acute glomerulonephritis. *J. Exp. Med.* **186**, 1853–1863 (1997).
30. van Spraid, A. B. et al. Mac-1 (CD11b/CD18) is essential for Fcg receptor-mediated neutrophil cytotoxicity and immunologic synapse formation. *Blood* **97**, 2478–2486 (2001).
31. Devi, S. et al. Multiphoton imaging reveals a new leukocyte recruitment paradigm in the glomerulus. *Nat. Med.* **19**, 107–112 (2013).
32. Rosetti, F. et al. Human lupus serum induces neutrophil-mediated organ damage in mice that is enabled by Mac-1 deficiency. *J. Immunol.* **189**, 3723–3728 (2012).
33. Fagerholm, S. C., MacPherson, M., James, M. J., Sevier-Guy, C. & Lau, C. S. The CD11b-integrin (ITGAM) and systemic lupus erythematosus. *Lupus* **22**, 657–663 (2013).
34. Williams, T. E., Nagarajan, S., Selvaraj, P. & Zhu, C. Concurrent and independent binding of Fcgamma receptors IIa and IIIb to surface-bound IgG. *Biophys. J.* **79**, 1867–1875 (2000).
35. Su, K. et al. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. *J. Immunol.* **178**, 3272–3280 (2007).
36. Pagan, J. D., Kitaoaka, M. & Anthony, R. M. Engineered sialylation of pathogenic antibodies in vivo attenuates autoimmune disease. *Cell Cell* **172**, 564–577 (2018).
37. Weber, K. S., York, M. R., Springer, T. A. & Klickstein, L. B. Characterization of lymphocyte function-associated antigen 1 (LFA-1)-dependent T cell lines: the alphaL and beta2 subunits are interdependent for cell surface expression. *J. Immunol.* **158**, 272–279 (1997).
38. Wright, S. D. & Meyer, B. C. Phorbol esters cause sequential activation and allosteric and interaction with ligands under force. *J. Biol. Chem.* **272**, 3057–3066 (1997).
39. Williams, T. E., Nagarajan, S., Selvaraj, P. & Zhu, C. Concurrent and independent binding of Fcgamma receptors IIa and IIIb to surface-bound IgG. *Biophys. J.* **79**, 1867–1875 (2000).
40. Su, K. et al. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. *J. Immunol.* **178**, 3272–3280 (2007).
41. Pagan, J. D., Kitaoaka, M. & Anthony, R. M. Engineered sialylation of pathogenic antibodies in vivo attenuates autoimmune disease. *Cell Cell* **172**, 564–577 (2018).
42. Weber, K. S., York, M. R., Springer, T. A. & Klickstein, L. B. Characterization of lymphocyte function-associated antigen 1 (LFA-1)-deficient T cell lines: the alphaL and beta2 subunits are interdependent for cell surface expression. *J. Immunol.* **158**, 272–279 (1997).
43. Wright, S. D. & Meyer, B. C. Phorbol esters cause sequential activation and allosteric and interaction with ligands under force. *J. Biol. Chem.* **272**, 3057–3066 (1997).
44. Wright, S. D. & Meyer, B. C. Phorbol esters cause sequential activation and allosteric and interaction with ligands under force. *J. Biol. Chem.* **272**, 3057–3066 (1997).
45. Williams, T. E., Nagarajan, S., Selvaraj, P. & Zhu, C. Concurrent and independent binding of Fcgamma receptors IIa and IIIb to surface-bound IgG. *Biophys. J.* **79**, 1867–1875 (2000).
46. Su, K. et al. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. *J. Immunol.* **178**, 3272–3280 (2007).
47. Pagan, J. D., Kitaoaka, M. & Anthony, R. M. Engineered sialylation of pathogenic antibodies in vivo attenuates autoimmune disease. *Cell Cell* **172**, 564–577 (2018).
48. Weber, K. S., York, M. R., Springer, T. A. & Klickstein, L. B. Characterization of lymphocyte function-associated antigen 1 (LFA-1)-deficient T cell lines: the alphaL and beta2 subunits are interdependent for cell surface expression. *J. Immunol.* **158**, 272–279 (1997).
49. Wright, S. D. & Meyer, B. C. Phorbol esters cause sequential activation and allosteric and interaction with ligands under force. *J. Biol. Chem.* **272**, 3057–3066 (1997).
50. Williams, T. E., Nagarajan, S., Selvaraj, P. & Zhu, C. Concurrent and independent binding of Fcgamma receptors IIa and IIIb to surface-bound IgG. *Biophys. J.* **79**, 1867–1875 (2000).
51. Su, K. et al. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. *J. Immunol.* **178**, 3272–3280 (2007).
52. Subedi, G. P., Falconer, D. J. & Barb, A. W. Carbohydrate-polypeptide contacts in the antibody receptor CD16A identified through solution NMR spectroscopy. *Biochemistry* **56**, 3174–3177 (2017).
53. Zhang, L. & Plow, E. F. Identification and reconstruction of the binding site within alphaMbeta2a for a specific and high affinity ligand, NIF. *J. Biol. Chem.* **272**, 17558–17564 (1997).
54. We thank Drs. Dmitry Solovjov and Edward Plow (Lerner Research Institute, The Cleveland Clinic, Cleveland, OH) for providing us GST-
55. Acknowledgements

We thank Drs. Dmitry Solovjov and Edward Plow (Lerner Research Institute, The Cleveland Clinic, Cleveland, OH) for providing us GST-al domain and Dr. Solovjov for valuable discussions, and Pragya Manandhar (Dept. of Biology and Biochemistry,
University of Houston) for providing us additional αI-domain. The work was supported by R01 HL065095, R21 AI136527, R01 DK099507, and the Lupus Distinguished Innovator award (Lupus Research Alliance) (T.M.), T32 AI074549 (G.S.), R01 AI124680 (C. Z.), NIAMS DP2AR068272 and the Lupus Research Alliance (R.A.) and R03 AI139651 (M.S.). FLIM studies were performed at the Massachusetts General Hospital Molecular Imaging Core, supported by NIH grant S10RR027931 to Roy J. Soberman, M.D.

Author contributions
G.S., K.O. and F.R. generated and characterized the Jurkat cell lines and conducted the flow and static adhesion and FLIM assays, X.C. made the cDNA mutants, Y.C and A.R. generated the BFP data, R.V. evaluated interactions of recombinant FcγRIIA and Mac-1 αI domain, N.T. conducted the in vivo mouse model, N.W. generated the glycosylation profiles for human neutrophil and Jurkat FcγRIIA and Jurkat FcγRIIIB, M.S. generated the structural models, S.H. provided blood from patients with LAD1, S.T. helped with mouse experiments, T.M., R.A., M.S. and C.Z. conceived and supervised the study.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-07506-1.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018