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Human Lupus Serum Induces Neutrophil-Mediated Organ Damage in Mice That Is Enabled by Mac-1 Deficiency

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Systemic lupus erythematosus (SLE) is a chronic, multiorgan inflammatory autoimmune disorder associated with high levels of circulating autoantibodies and immune complexes. We report that passive transfer of human SLE sera into mice expressing the uniquely human FcγRIIA and FcγRIIB on neutrophils induces lupus nephritis and in some cases arthritis only when the mice additionally lack the CD18 integrin, Mac-1. The prevailing view is that Mac-1 on macrophages is responsible for immune complex clearance. However, disease permitted by the absence of Mac-1 is not related to enhanced renal immune complex deposition or in situ C1q/C3 complement activation and proceeds even in the absence of macrophages. Instead, disease is associated with increased FcγRIIA-induced neutrophil accumulation that is enabled by Mac-1 deficiency. Intravital microscopy in the cremasteric vasculature reveals that Mac-1 mitigates FcγRIIA-dependent neutrophil recruitment in response to deposited immune complexes. Our results provide direct evidence that human SLE immune complexes are pathogenic, demonstrate that neutrophils are primary mediators of end organ damage in a novel humanized lupus mouse model, and identify Mac-1 regulation of FcγRIIA-mediated neutrophil recruitment as a key step in development of target organ damage. The Journal of Immunology, 2012, 189: 000–000.
FcγRIIB selectively on neutrophils of Fcγ-chain–deficient mice (IIA⁺γ⁻, IIA⁺IIIb⁺γ⁻) restored susceptibility of γ⁻ animals to progressive anti-glomerular basement membrane (GBM) nephritis and K/B×N serum-induced arthritis (13, 14). Although both human FcγRs promoted neutrophil accumulation, only FcγRIIA alone induced tissue injury most likely through its established ability to promote neutrophil cytotoxic functions (13).

Difficulty in understanding the underlying mechanisms of SLE arises from the multifactorial influences on disease course, including environmental, infectious, and hormonal factors, the association of lupus with abnormalities at all levels of the immune system, and the clinical heterogeneity of the disease (7). The association of lupus with abnormalities at all levels of the immune system, and the clinical heterogeneity of the disease (7). The proportion of FcγRIIb receptors on neutrophils determines neutrophil interactions with ICs.

Electron microscopy. Kidney samples were taken 10 d after injection of SLE sera and processed using standard techniques.

Generation of a K562 stable cell line and analysis of binding to ICAM-1

The R77H mutation was introduced into human CD11b DNA by standard PCR techniques. K562 cells were transfected with WT or mutant (R77H) CD11b and CD18 plasmids by electroporation and confirmed by sequencing. Single-cell sorting was used to select CD11b positive cells. Cells were cultured in RPMI 1640 media supplemented with 10% heat-inactivated FBS, under constant selection using G418 (500 μg/ml; Invirgen). CD11b expression was assessed by FACS analysis using anti-human CD11b-PE–labeled Ab (BD Pharmingen). To evaluate K562 cell binding to ICAM-1-Fc, cells were preincubated with 1 μM MnCl₂ for 10 min in HEPES buffer and drawn across a coverslip containing immoderately ICAM-1-Fc (15 μg/ml; R&D Systems) under 0.1 dynes/cm² shear flow. The number of cells bound in four different fields was quantified every 2 min over a 10-min period.

IgG depletion and quantification of human IgG

SLE serum was incubated with protein A/G-coated agarose beads (Thermo Scientific) or control agarose beads. Depletion was determined by measuring human IgG by ELISA (Bethyl Laboratories). Human ICs were quantified in human and mouse serum using an ELISA kit with C1q as the capture element (ALPCO Immunoassays). Due to cross-reactivity with mouse IgG, an anti-human HRP Ab (Bethyl Laboratories) was used as the secondary Ab for the quantification of human ICs in murine serum samples.

Macrophage depletion using cladronate liposomes

Mice were given an i.p. injection of 500 μl liposomes containing cladronate or PBS (23) on day −5 before the first SLE injection, followed by 200 μl every 5 d. Tissue was harvested at day 10 or 14 after SLE sera injection. Macrophage depletion was confirmed by immunohistochemistry of kidneys and spleen using anti-F4/80 Ab.

Materials and Methods

Mice

Fcγ-chain–deficient mice, human FcγRIIA and FcγRIIB-expressing γ-chain–deficient mice (IIA⁺γ⁻ or IIA⁺IIIb⁺γ⁻), Mac-1–deficient mice (Mac-1⁻), and CD18−/− deficient mice (CD18−/−) were as described (13, 15–17). Mac-1–deficient mice were bred with IIA⁺γ⁻ or IIA⁺IIIb⁺γ⁻ mice to generate IIA⁺γ⁻Mac-1⁻ and IIA⁺IIIb⁺γ⁻Mac-1⁻ animals or with wild-type (WT) mice to generate IIA⁺γ⁻Mac-1⁻ and IIA⁺γ⁺/⁺ mice. Mice were bred in a specific pathogen-free facility. Age- and gender-matched mice 8–12 wk old were used in all experiments. The National Institutes of Health guidelines on animal care were followed.

Functional assessment of renal damage. Spot urine samples were collected, and urine albumin and creatinine were evaluated by ELISA (Bethyl Laboratories) and a chemical assay (Cayman Chemical), respectively (13), and expressed as a ratio of urine albumin to creatinine.

Clinical scoring of arthritis. Mice were evaluated every other day after induction of disease. Inflammation of each limb was scored, as reported previously (19): 0, no evident inflammation; 1, redness or swelling of 1 toe; 2, redness or swelling of 1 toe; 3, ankle or tarsal-metatarsal involvement; 4, redness or swelling of the entire paw.

Histological assessment of tissue injury. Tissue sections were blindly evaluated. Histological score included endocapillary proliferation, leukocyte infiltration, and crescents, as previously described (20). For joint lesions, histological scores reflected leukocyte infiltration, synovial thickening, and cartilage and bone erosion.

Histological studies

Kidneys were fixed in formalin and paraffin embedded, or frozen in OCT medium, and 5-μm sections were prepared. Tissue sections were fixed in 4% paraformaldehyde and decalcified with modified Kristensen’s solution. After dehydration, the tissues were embedded in paraffin and 5-μm sections were made (21). Immunohistochemistry. For kidneys, periodic acid-Schiff, H&E, and dichloroacetate esterase (to identify neutrophils) on paraffin-embedded sections were performed, as described (22). Neutrophils in 100 glomerular cross-sections were quantified and presented as neutrophils per glomerular cross-section. Immunohistochemistry on frozen sections was performed using a two-layer peroxidase method. Sections were immunostained with anti-F4/80 for macrophages (BioLegend), anti-CD3 (Serotech) for T cells, and anti-C1q (Hyclut Biotech) for C1q deposition, and counterstained with Gill Hematoxylin No. 2 (NewComerSupply). Tissue area occupied by macrophages or glomerular C1q deposition in four ×20 consecutive fields was quantified using ImagePro. Sections of paraffin-embedded tissues were stained with H&E, and immunohistochemistry was performed with anti–NIMP-R14 (Abcam) for neutrophil quantification, anti-F4/80 (BioLegend) for macrophages, anti-CD3 (Serotech) for T cells, or anti-human IgG (Invitrogen). The total number of neutrophils in a toe cross-section was counted and divided by the total tissue area in each section, using ImagePro.

Immunofluorescence. Human and mouse IgG deposition were evaluated on frozen sections using anti-human IgG or anti-mouse IgG (Invitrogen) Alexa fluor 488–conjugated Abs, respectively. Deposition of C3 was evaluated using a FTC-conjugated anti-C3 Ab (ICN/CAPPEL). Images of at least 15 glomeruli per mouse were captured, and glomerular fluorescence intensity was measured using the acquisition and analysis software Metamorph (Molecular Devices). To evaluate human IgG subclasses, immunofluorescence was performed using anti-human IgG1, IgG2, IgG3, and IgG4 Abs (The Binding Site), followed by an anti-sheep DyLight-488–conjugated Ab (Jackson ImmunoResearch Laboratories).

Electron microscopy. Kidney samples were taken 10 d after injection of SLE sera and processed using standard techniques.

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**Intravital microscopy evaluation of reverse passive Arthus reaction in the cremaster muscle**

Rabbit IgG anti-BSA Ab (200 μg/300 μl; Sigma-Aldrich, St. Louis, MO) was injected intracutaneously, followed by an i.v. injection of BSA (300 μg/100 μl; Sigma-Aldrich). After 3 h, leukocyte recruitment in the cremaster of anesthetized mice was evaluated by intravital microscopy, as previously described (13). Four venules per mouse were analyzed. Leukocyte rolling velocities were measured by tracking single leukocytes (10/venule) over several frames and calculating the distance moved per unit time (μm/s). Adherent leukocytes were defined as cells remaining stationary for 30 s and were expressed as the number of cells/mm² venule.

MIP-2 was locally applied, as previously described (24). Briefly, injections were done using a beveled glass pipette of 1 mm outer diameter mounted on a manual micromanipulator (MM-33; Warner Instruments, Hamden, CT). The tip was filled with 100 nmol/L MIP-2 (U.S. Biologicals). The pipette was placed, and the number of rollers across a perpendicular line and the number of adherent cells (Aₚ) in a 200-μm segment of the venule were recorded over a 1-min period. Next, air pressure was applied to the pipette, to trigger the injection of MIP-2 (<1 μl). Successful injection was verified by the presence of swelling of the interstitial tissue surrounding the pipette tip. The process of injection and the following minute were recorded. The number of cells that were adherent after MIP-2 injection (Aₜ) was evaluated. The Δ adherent cells, which is the percentage of rollers that adhered in response to MIP-2 injection, was calculated using the following equation: [(Aₚ – Aₜ)/number of rollers] × 100.

**Statistical analysis**

Data are expressed as mean ± SEM. For proteinuria data, the line represents the median of the group. Differences were determined by Mann-Whitney U test. For general analysis, one-way ANOVA was used for proteinuria data and two-way ANOVA was used for the cellular infiltration data. When significant differences were shown, data were subjected to Mann-Whitney U test or Bonferroni test, respectively, for comparison between two mouse strains. The p values <0.05 were considered significant.

**Results**

**Human SLE serum induces nephritis in mice that express neutrophil human FcγRs and lack Mac-1**

Human SLE serum was obtained from patients that had active disease (SLE disease activity index ≥6) and no clinical evidence of infection. Gender- and race-matched healthy individuals served as controls. Mice preimmunized with human IgG/CFA received two i.v. injections of SLE sera at days 0 and 2. Nephritis was monitored by analyzing albumin leakage in the urine over a 21-d period. SLE serum from patient A caused marked proteinuria in mice that expressed human FcγRs (hFcγRs) only when these mice additionally lacked Mac-1 (IIA*’/Mac-1–/– and IIB*’/Mac-1–/–) (Fig. 1A). Proteinuria peaked at day 14 (Supplemental Fig. 1A). Mac-1–/deficient mice expressing FcγRIIIA alone, without FcγRIIBb, exhibited significant proteinuria, suggesting that this hFcγR was sufficient for disease development (Fig. 1A). No proteinuria was observed in Mac-1–sufficient mice expressing hFcγRs (IIA*’/Mac-1–/– or IIB*’/Mac-1–/–), Mac-1–/deficient mice without hFcγRs (IIA*’/Mac-1–/–), or WT animals (Fig. 1A). Surface expression of human FcγRs and the sister CD18 integrin, LFA-1, was similar in Mac-1–/deficient and sufficient groups (Supplemental Fig. 1B). Any effect of γ-chain deficiency superimposed on Mac-1 deficiency on disease susceptibility was ruled out as SLE serum induced nephritis in mice that express hFcγRIIIA, lack Mac-1, and were γ-chain sufficient (Fig. 1B).

Mouse anti-human IgG was detected in the serum (Supplemental Fig. 1C) and in the kidney (Fig. 1C) after SLE sera transfer, but this was much less compared with that in WT mice subjected to anti-GBM (nephrotic) nephritis (Fig. 1C), a widely used model of immune-mediated disease induced by preimmunization with rabbit IgG/CFA, followed by transfer of rabbit anti-GBM serum (25). Importantly, mouse anti-human IgG levels in both the serum and renal tissue were similar in disease-susceptible and nonsusceptible mice (Supplemental Fig. 1C, Fig. 1C). Although a role for mouse anti-human IgG and/or ICs formed from human IgG reacting with Ags in the mouse serum cannot be formally ruled out, it is unlikely to be critical for disease development for the following two reasons. First, SLE sera induced nephritis in the absence of preimmunization with CFA/human IgG (data not shown), albeit disease was more variable. Second, human FcγRs, and thus by inference human IgG, are required for development of nephritis. In the anti-GBM murine model, rabbit IgG triggers a robust murine anti-rabbit reaction; nonetheless, tissue injury is largely independent of the humoral response (26). We propose that, as is known for other autoimmune models, activation of the innate immune response by CFA in our SLE model may be important independent of the adaptive immune response (27).

**Histopathological and electron microscopy analyses of renal tissue from human SLE sera-treated mice were undertaken.**

Concurrent with proteinuria, glomerular damage and inflammation were observed only in hFcγRγ’−/Mac-1−/− animals (Fig. 1D, 1E) and were associated with mesangial deposits of ICs (Fig. 1E). Together these data suggest that Mac-1 deficiency enables SLE-induced kidney damage. This may have relevance to human disease because the R77H polymorphism in the ITGAM gene (that encodes the CD11b chain of Mac-1) associated with SLE abrogates the capacity of Mac-1 expressed in a cell line, to bind its ligand ICAM-1 (Fig. 2).

**Pathogenicity of SLE serum depends on the IgG fraction and its capacity to deposit in glomeruli**

Sera collected from different SLE patients were tested in susceptible IIA*’IIIB*’/Mac-1−/− mice. Four of six SLE sera induced nephritis, whereas sera from five healthy controls (normal serum) or heat-aggregated human IgG (surrogate of ICs) failed to induce disease (Fig. 3A). Moreover, four of five sera from SLE patients at an independent clinical center induced nephritis compared with normal controls (Supplemental Fig. 1D). Notably, nephritis was observed with multiple SLE serum samples from the same patient when treatment and disease activity varied, but circulating ICs remained elevated. IgG depletion of SLE serum A abrogated its nephritis-inducing capacity (Fig. 3B), which suggests that ICs in the sera are required for disease development (Fig. 1A).

The sera that caused disease, henceforth referred to as pathogenic sera (e.g., SLE sera A and E), were associated with glomerular deposits of human IgG that exhibited a granular pattern characteristic of ICs (Fig. 3C). In contrast, no human IgG deposition was observed when nonpathogenic sera were injected; these included SLE sera D and F and sera from healthy controls (Fig. 3C). All four human IgG isotypes, IgG 1–4, were observed in the glomeruli, predominantly as deposits in the mesangium (Fig. 3D). Specificity of the Abs was confirmed by analyzing their reactivity against purified human IgGl–4 in Western blots (data not shown). In the case of serum A, focal tubular basement membrane deposition of IgG was also detected (Fig. 3D), which is a pattern observed in human lupus nephritis. SLE sera pathogenicity (i.e., capacity of human ICs to deposit in glomeruli) did not correlate with the amount of ICs or IgG present in the sera (Supplemental Table 1A), nor did it correlate with the patient type or lupus manifestations (Supplemental Table 1B).

**The susceptibility conferred by Mac-1 deficiency is not due to defects in IgG clearance**

Levels of circulating (Fig. 4A) and deposited human IgG-ICs (Fig. 4B) as well as glomerular complement C1q and C3 deposition
Fig. 4B) were comparable between hFcγR-expressing mice that were Mac-1 sufficient (IIA+/IIIB+/IIIC2/2) or deficient (IIA+/IIIB+/IIIC2/2 Mac-12/2), as were peripheral blood neutrophil and leukocyte counts (Supplemental Table 1C).

Passive transfer of human SLE serum induces arthritis in mice

Notably, two of the pathogenic SLE sera, A (Fig. 5) and E (data not shown), caused arthritis in mice that affected primarily small joints, namely the distal interphalangeal, and were evident at day 4. Patient A had clinical manifestations of arthritis, and patient E did not have arthritis, but presented with Raynaud syndrome. There was no correlation between human symptoms and arthritis development, as some sera from patients who clinically presented with nephritis and arthritis did not induce arthritis in mice (data not shown). Moreover, unlike nephritis, development of arthritis was more variable with different sera samples. As in the case of nephritis, hFcγR-expressing mice lacking Mac-12/2 developed arthritis (Fig. 5A). Histopathological analyses revealed intense joint inflammation with cellular infiltration associated with bone and cartilage destruction (Fig. 5B). Significant deposition of human IgG-IC within synovial blood vessels was observed in both disease-susceptible and nonsusceptible mice (Fig. 5C). Neutrophils were abundant in the lesions (Fig. 5D), whereas macrophages and T cells were not detected (data not shown).

Marked glomerular neutrophil infiltration is observed in susceptible mice, whereas macrophages do not contribute to disease

Susceptible mice (hFcγR+γ−/−Mac-1−/−) exhibited abundant glomerular neutrophil infiltration at day 10 that significantly exceeded that in nonsusceptible mice (Fig. 5A). In contrast, a mild interstitial macrophage and T cell infiltration was observed in both groups of mice (Fig. 6A). Thus, Mac-1 deficiency appears to selectively enable neutrophil accumulation. To distinguish whether the Mac-1-expressing cell responsible for resistance to nephritis corresponded to circulating (i.e., monocytes or neutrophils) or

FIGURE 1. SLE sera induces nephritis in mice that express hFcγR and lack Mac-1. (A) Mice received two i.v. injections of SLE sera A on days 0 and 2, and urine albumin (normalized to creatinine) at day 14 was evaluated. The dashed line indicates the mean of proteinuria in mice not given SLE sera. Each data point represents one mouse, and the solid line is the median of the group. (B) Mice expressing the FcγRIIA in the presence of the γ-chain (IIA+γ+), and the same that are additionally Mac-1-deficient (IIA+γ+Mac-1−/−) were injected with SLE sera A and albuminuria was evaluated at days 7 and 14. Albuminuria in IIA+γ−/−Mac-1−/− mice given SLE sera was analyzed in parallel for comparison. Each dot represents one animal, and the line indicates the median of the group. ns, not significant. (C) Murine anti-human IgG was quantified in renal tissue by immunofluorescence in indicated transgenic mice that were untreated (Unt) or 21 d following SLE sera A transfer (SLE). WT mice given rabbit anti-GBM injection (WT aGBM) served as a positive control. Mean ± SEM are graphed. Dashed line is the average of pixel intensity in the Unt mice. (D) Representative images of renal sections (day 21) stained with H&E or periodic acid-Schiff (PAS). The arrow indicates a glomerular crescent that was observed in ~5% of the glomeruli. A histological score was given, and mean ± SEM was graphed (n ≥ 5 mice per group). (E) Electron microscopy of glomeruli of mice given SLE sera: (i) IIA+IIIB+γ−/− mice had normal glomerular architecture; (ii) and (iv) IIA+IIIB+γ−/−Mac-1−/− mice exhibited mesangial expansion with increased cellularity (ii), neutrophils within capillary loops (iii) (×4,730), mesangial electron-dense deposits (*), and lysosomal vacuoles containing protein reabsorption droplets (arrows) (iv) typically observed during proteinuria (×11,100). *p < 0.05, **p < 0.01, ***p < 0.001. en, Endothelial; m, mesangial cell; n, neutrophils; p, podocyte.
resident cells, we generated bone marrow chimeras. Bone marrow from IIAγ−/− or IIAγ−/−Mac-1−/− mice was transferred into lethally irradiated γ−/−Mac-1−/− or γ−/− mice, respectively (i.e., IIAγ−/−→γ−/−Mac-1−/− and IIAγ−/−Mac-1−/−→γ−/−), and 3 mo later SLE serum was administered. IIAγ−/−Mac-1−/− bone marrow provided susceptibility to nephritis when transferred into nonsusceptible γ−/− mice, suggesting that a lack of Mac-1 on hFcγRIIA-expressing circulating cells allows the development of disease (Supplemental Fig. 2A). Immunohistochemistry in tissue of γ−/−Mac-1−/− recipients of IIAγ−/− bone marrow revealed Mac-1−/−positive macrophages in the renal interstitium following human SLE serum transfer, suggesting that SLE serum induces renal infiltration of blood monocytes and their subsequent differentiation into macrophages (Supplemental Fig. 2A). To determine whether monocytes/macrophages contribute to renal injury, these populations were depleted prior to SLE serum transfer by administering clodronate liposomes, which are internalized by these cells and leads to their apoptosis (Supplemental Fig. 2B). Macrophage-depleted IIAγ−/−Mac-1−/− mice remained highly susceptible to nephritis (Fig. 6B), suggesting that macrophages do not significantly contribute to renal injury. Moreover, macrophage depletion in disease-non-susceptible (IIAγ−/−) mice did not result in proteinuria (Fig. 6C) or glomerular neutrophil infiltration (data not shown) at day 10, suggesting that Mac-1 deficiency in monocytes/macrophages does not enable disease.

Mac-1 deficiency modifies the response of neutrophils toward tissue-deposited ICs in vivo

To elucidate the mechanism(s) by which Mac-1 regulates FcγRIIA-mediated neutrophil accumulation, we conducted intravital microscopy of the cremaster muscle that allows the real-time visualization of neutrophil behavior within the vessel wall. The reverse passive Arthus (RPA) reaction, induced by the i.v. administration of soluble BSA and an intrascrotal injection of anti-BSA, results in IC deposition within and outside the cremasteric vessels (13, 28). The RPA induces neutrophil rolling that is P- and E-selectin dependent (28), whereas the slowing of the rolling velocity and adhesion is dependent on murine FcγRs (29) and FcγRIIA (13). Following induction of the RPA, neutrophils in the venules of IIAγ−/−Mac-1−/− mice rolled significantly slower than in IIAγ−/− mice (Fig. 7A). This was not a result of differences between the two groups in expression of LFA-1 on circulating neutrophils or neutrophils recruited to the cremaster (Supplemental Fig. 2C). The slow rolling required FcγRIIA, as it was not observed in γ−/− Mac-1−/− and γ−/− mice (Fig. 7B). The downmodulatory effect of Mac-1 was selective for ICs, as the rolling velocity induced by an intrascrotal injection of TNF-α was actually increased in IIAγ−/−Mac-1−/− mice compared with IIAγ−/− mice (data not shown), as previously described (30). In the RPA, the observed slower neutrophil rolling in IIAγ−/−Mac-1−/− mice was not associated with an increase in the number of adherent cells compared with IIAγ−/− mice (Fig. 7C). E-selectin-mediated activation of LFA-1 has been shown to slow neutrophil rolling velocity, and in turn lead to efficient firm adhesion only in response to a local chemotactrat stimulus, MIP-2 (24). Accord-
Interestingly, the local microinjection of MIP-2 favored adhesion of neutrophils in IIαγ−/−Mac-1−/− compared with IIαγ−/− animals subjected to the RPA (Fig. 7D). IC induced slow rolling, and MIP-2–induced neutrophil adhesion was most likely LFA-1 dependent, as these steps were markedly diminished in CD18-deficient mice (Fig. 7E, 7F).

In summary, FcγRIIA-dependent slow rolling, which correlates with efficient leukocyte adhesion in response to MIP-2, is regulated by Mac-1, with a deficiency in this integrin, resulting in a further decrease in rolling velocity and subsequent enhanced responsiveness of neutrophils to a local chemotactic stimulus.

Discussion

In this study, we show in a novel lupus model that IC deposition and organ damage are not inextricably linked, but that additional genetic factors dictate the propensity for target organ damage. In particular, we demonstrate that the neutrophils’ response to deposited ICs and subsequent tissue injury, following human lupus sera transfer, is modulated by a functional association between an integrin and an IgG receptor. The role of Mac-1 in attenuating FcγRIIA-mediated neutrophil recruitment in the context of deposited ICs is unanticipated from previous work that has established Mac-1 largely as a positive regulator of neutrophil influx and inflammation (8, 31, 32). Our data predict that the R77H Mac-1 variant, which leads to reduced binding to its ligands, may have functional consequences for SLE patients and offer a mechanism by which Mac-1 dysfunction may contribute to end organ injury in SLE.

Disease susceptibility permitted by the absence of Mac-1 was not related to alterations in IC handling presumably by macrophages. Moreover, Mac-1 on macrophages and indeed macrophages themselves did not influence disease susceptibility. Thus, this cell type, abundant in SLE lesions (33), is not essential for target organ injury in our model at least in the time frame of our experiments. Instead, Mac-1 deficiency most likely predisposes to disease development due to unrestrained FcγRIIA-mediated neutrophil accumulation in the kidney. The reported physical interaction of Mac-1 with human FcγRs on the cell surface (34) and the sharing of intracellular ITAM-based signaling cascades by these two receptors (35) led us to the intriguing possibility that Mac-1 on neutrophils, in cis, modulates neutrophil FcγRIIA activity and function.

FcγRIIA on neutrophils clearly has the capacity to induce tissue injury in vivo (13, 14). However, in the context of SLE-IC deposits in the kidney and joints, the regulation of FcγRIIA-mediated neutrophil recruitment appears to be a key step in conferring disease susceptibility. Recent work suggests that binding of FcγRIIA to ICs and FcγRIIA function may be regulated. In vitro, the G protein-coupled receptor agonist C5a and fmlp enhance (14, 36), whereas coengagement of FcγRIIB inhibits (37) FcγRIIA function. How is FcγRIIA regulated by Mac-1? Clues were provided by intravital microscopy. Slowing of the velocity of rolling cells improves their efficiency of firm adhesion (30), and, in the RPA, FcγRIIA plays an important role in mediating slow rolling and leukocyte arrest (13). Additionally, CD18 integrins are required as slow rolling, and MIP-2–mediated adhesion in the RPA is abrogated in CD18-deficient mice. We postulate that FcγRIIA promotes the activation of LFA-1. A deficiency in Mac-1 heightens FcγR activity toward ICs and subsequent LFA-1 activation that in turn leads to further deceleration of the rolling velocity and increased responsiveness to MIP-2. Mac-1 may be responsible for mitigating FcγRIIA-mediated signaling or limiting FcγRIIA mobility or clustering, the molecular details of which require further investigation. Human FcγRIIA appears to functionally interact with murine Mac-1 in cis, suggesting that this interaction uses conserved features of human and mouse Mac-1.

The observed decrease in neutrophil accumulation in Mac-1-deficient mice in other models of IC-mediated disease, including acute anti-GBM, thrombotic glomerulonephritis, and bullous pemphigoid (22, 38, 39), suggests differential roles for Mac-1 in distinct models of IC-based diseases. Complex biological circuits predict dual and sometimes opposing roles for the same receptor that is cell type and context dependent. For example, incomplete phosphorylation of the ITAM following engagement of B or TCRs by low affinity/valency ligands favors inhibitory over activating signaling by these immune receptors (40). Moreover, unsustained FcγRII clustering markedly inhibits, whereas sustained FcγRII aggregation promotes cell activation through differential recruitment of the tyrosine kinase Syk versus an inhibitory protein phosphatase Src homology region 2 domain-containing phospha-
We posit that Mac-1 relays similar context-dependent inhibitory and activating signals, a hypothesis that is supported by the finding that, although the R77H Mac-1 polymorphism is a susceptibility factor for lupus (9), it does not predispose to rheumatoid arthritis (43), another IC-mediated disease. Interestingly, FcγRIIIB may also play context-dependent roles in IC-mediated inflammation. Whereas low expression of FcγRIIIB is associated with SLE (44), high FcγRIIIB levels increase susceptibility to anti-neutrophil cytoplasmic Ab-associated systemic vasculitides, a disease associated with in situ rather than soluble ICs (45, 46). This concept is recapitulated in our mouse models. Human SLE serum-induced nephritis was reduced in mice expressing both human FcγRIIA and FcγRIIIB versus FcγRIIA alone, which contrasts with the greater proteinuria following anti-
GBM nephritis in mice expressing both hFcγRs compared with mice expressing FcγRIIA alone (13). Our studies suggest that neutrophils are the primary cellular link between IgG and target organ damage in our model. Neutrophils are present in renal SLE lesions (47, 48), precede macrophage infiltration (47), and are appreciated as a sign of disease severity (49). Importantly, recent studies identified a subset of neutrophils in SLE patients that synthesize type I IFNs, induce endothelial cell damage (50), release neutrophil extracellular traps that are potentially autoantigenic, and drive IFN-α production by dendritic cells (51, 52). SLE sera transfer results in the development of lesions that recapitulate several features of lupus nephritis. The mice obviously lack the imbalances in the adaptive immune response and therefore chronicity of the disease. However, like SLE patients, in which development of lupus nephritis is not associated with autoantibodies of singular IgG specificity (53) a spectrum of IgG1–4 subtypes was found deposited in the glomerulus with the capacity of SLE sera to induce proteinuria being directly correlated with their ability to deposit in the glomerulus. Histological

FIGURE 6. Disease in susceptible mice is associated with neutrophil accumulation; macrophages do not participate in disease development. (A) Kidneys were harvested from mice at day 21 after injection of SLE sera A. Dotted line represents the mean value obtained in susceptible mice given normal human serum and harvested at day 10 (top panel) or day 14 (middle and bottom panels). Neutrophil, macrophage, and T cell infiltration were quantified by immunohistochemistry. Mice treated with anti-GBM serum are included as a positive control for indicated leukocyte infiltration. Mean ± SEM is shown. *p < 0.05, **p < 0.01 compared with IIA⁺IIIB⁺γ⁺ and IIA⁺γ⁺ Mac-1⁺ mice (n = 13 venules in 3 mice per group). Cumulative histograms of rolling velocities are shown. (B) The number of adherent neutrophils in mice analyzed in (A) was determined. (C) Mice were subjected to the RPA, the cremaster was prepared for intravital microscopy, and a local injection of MIP-2 was given as depicted in the diagram. The increase in neutrophil adhesion following injection of MIP-2 was measured (D) of adherent cells in IIA⁺γ⁺ and IIA⁺γ⁺ Mac-1⁺ mice was calculated (n = 3 venules in 6–7 mice per group). Cumulative histogram of neutrophil rolling velocities of WT and CD18⁺⁻ mice subjected to the RPA, and (F) of adherent cells upon MIP-2 injection are shown (n = 6 venules in 3 mice per group). For (C), (D), and (F), mean ± SEM are graphed. **p < 0.01, ***p < 0.001.

FIGURE 7. Mac-1 deficiency slows the rolling velocity and increases the chemokine-induced adhesion of neutrophils following the RPA reaction. The RPA was induced in the cremaster, and, 3 h later, the mice were prepared for intravital microscopy and neutrophil rolling velocity was measured in hemodynamically similar venules of (A) IIA⁺γ⁺ Mac-1⁻ and IIA⁺γ⁺ mice (n = 33 venules in 6 mice per group) and (B) γ⁺⁻ Mac-1⁻ and γ⁺⁻ mice (n = 13 venules in 3 mice per group). Cumulative histograms of rolling velocities are shown. (C) The number of adherent neutrophils in mice analyzed in (A) was determined. (D) Mice were subjected to the RPA, the cremaster was prepared for intravital microscopy, and a local injection of MIP-2 was given as depicted in the diagram. The increase in neutrophil adhesion after local MIP-2 injection (Δ of adherent cells) in IIA⁺γ⁺ and IIA⁺γ⁺ Mac-1⁻ mice was calculated (n = 15–16 venules in 6–7 mice per group). (E) Cumulative histogram of neutrophil rolling velocities of WT and CD18⁺⁻ mice subjected to the RPA, and (F) of adherent cells upon MIP-2 injection are shown (n = 6 venules in 3 mice per group). For (C), (D), and (F), mean ± SEM are graphed. **p < 0.01, ***p < 0.001.
features similar to human disease include mesangial hypercellularity and mesangial deposits (mainly IgG), C3 deposition, endothelial and extracapillary proliferation (crenulatons), and neutrophils. The lack of the interstitial component, thickening of the membrane or podocyte effacement, is likely because the immune deregulation in SLE patients is much more profound than in our model. Renal injury in our model is unlikely a consequence simply of an immune response against heterologous human IgG, as normal human serum, some SLE-patient serum, and heat-aggregated human IgG failed to induce nephritis and murine anti-human IgG Ab deposition in the glomeruli was not significant.

Indiscriminate immunosuppression remains the prevailing therapy in SLE with significant toxicity, and the expectations from therapeutic targeting of the aberrant immune system have remained unfulfilled (4). Our data in a humanized mouse model indicate that deposition of circulating autoantibodies is not sufficient for target organ damage. Rather, regulation of neutrophil FcγRIIA by Mac-1 fundamentally influences IC-mediated end organ damage. Moreover, our humanized passive SLE sera transfer model will aid in delineating additional mechanisms specifically driving lupus-induced tissue injury and serve as a preclinical platform to test new therapeutics targeted at preventing lupus end organ damage.

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Disclosures
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