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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 systemic, 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 cremaster 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: 3714–3723.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects multiple organs, including the kidney, joints, and skin, with development of nephritis being one of the most serious complications of this disorder. SLE is characterized by a loss of tolerance to self-Ags and high titers of circulating autoantibodies (1). In mice, deletion of the common...
Materials and Methods

Mice

Fcγ-chain–deficient mice, human FcγRIIA and FcγRIIB-expressing γ-chain–deficient mice (HIA-γ−/− or HIA-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+2/2 mice to generate IIA+2/2 or IIA+IIIB+2/2 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 Harvard Medical School Animal Care and Use Committee approved all procedures used in this study.

Passive transfer of human SLE sera or rabbit anti-GBM Ab

For the human SLE sera transfer model, mice were injected s.c. in both flanks with 2.5 μl human IgG (Jackson ImmunoResearch Laboratories) in CFA (Thermo Scientific and Difco) on day −3. On days 0 and 2, 200 μl sterile serum was injected i.v. For induction of nephrotoxic nephritis, mice were given rabbit IgG in CFA at day −3 in the footpad and rabbit αGBM antiserum at day 0, as described (18), and urine and kidneys were collected at day 21.

Human sera samples

Human serum samples were obtained from the Lupus Clinic at the Beth Israel Deaconess Medical Center or Instituto Nacional de Ciencias Medicas y de la Nutricion Salvador Zubiran. Patients with SLE were diagnosed according to the classification criteria of the American College of Rheumatology. The inclusion criteria for SLE patients were patients who fulfilled ≥4 of 11 American College of Rheumatology criteria for SLE and at the time of the first blood draw had active disease (i.e., SLE disease activity index ≥6) and were diagnosed with nephritis. Normal serum was obtained from healthy individuals matched for age, gender, and ethnicity. At the time of serum collection, all individuals showed no signs or symptoms of infection. Informed consent was obtained from all patients and healthy donors under a Beth Israel Deaconess Medical Center Institutional Review Board-approved protocol. Unless indicated, all experiments were performed using serum from SLE patient A.

Disease evaluation

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. Toe specimens were fixed in 4% paraformaldehyde and decalcified with modified Kristensen’s solution. After decalcification, the tissues were embedded in paraffin and 5-μm sections were made (21).

Immunohistochemistry. For kidneys, periodic acid–Schiff, H&E, and dichlororacetate 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 (Hydult 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 toes 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 FITC-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.

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; InvivoGen). 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 preactivated with 1 mM MnCl2 for 10 min in HEPES buffer and drawn across a coverslip containing immobilized ICAM-1-Fc (15 pg/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 clodronate liposomes

Mice were given an i.p. injection of 500 μl liposomes containing clodronate 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.
**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 $\geq 6$) 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+IIIB- and IIA+IIIB+Mac-1-/-) (Fig. 1A). Proteinuria peaked at day 14 (Supplemental Fig. 1A). Mac-1-deficient mice expressing FcγRIIIA alone, without FcγRIIIB, 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+IIIB+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 $\gamma$-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 $\gamma$-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 (nephrototoxic) 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 $\gamma$s 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. Congruent with proteinuria, glomerular damage and inflammation were observed only in hFcγR$\gamma$-/-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 IgG1–4 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
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 αGBM) 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.

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 αGBM) 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.

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. 6A). 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...
not significantly contribute to renal injury. Moreover, macrophage
susceptible to nephritis (Fig. 6B), suggesting that macrophages do
not contribute to renal injury, these

Macrophage-depleted IIA mouse was transfected into lethally irradiated γ/−/− Mac-1−/− or γ/−/− mice, respectively
(i.e., IIαγ−/− → γ/−/− Mac-1−/− and IIαγ−/− Mac-1−/− → γ−/−). bone marrow provided susceptibility to nephritis when transferred into nonsusceptible γ−/− mice, suggesting that a lack of Mac-1 on hFcRIIA-expressing circulating cells allows the development of disease (Supplemental Fig. 2A). Immunohistochemistry in tissue of γ−/−Mac-1−/− recipients of IIαγ−/− 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 IIαγ−/− 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-nonsusceptible (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, 128). 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 IIαγ−/− Mac-1−/− mice rolled significantly slower than in IIαγ−/− 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
responsiveness of neutrophils to a local chemotactic stimulus. A further decrease in rolling velocity and subsequent enhanced adhesion by Mac-1, with a deficiency in this integrin, resulting in efficient leukocyte adhesion in response to MIP-2, is regulated by 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 agonists C5a and fmlp enhance FcγRIIA-mediated neutrophil accumulation in the kidney. 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 is 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.

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 FcεRI clustering markedly inhibits, whereas sustained FcεRI aggregation promotes cell activation through differential recruitment of the tyrosine kinase Syk versus an inhibitory protein phosphatase Src homology region 2 domain-containing phospha-

![FIGURE 4. IC clearance and deposition are similar in susceptible and nonsusceptible mice. (A) Serum was collected from animals at intervals up to day 14 after SLE sera A transfer. Circulating human IgG (hIgG) and human IgG ICs (hICs) were evaluated, and the mean ± SEM were graphed. (B) Kidneys were harvested at day 14 after SLE sera A transfer, and renal sections were stained for hIgG, murine C1q, and complement C3. The mean fluorescence intensity (MFI; average pixel intensity ± SEM) for glomerular hIgG and C3 and the percentage of glomerular area positive for C1q (% area ± SEM) were determined and found to be equivalent in both groups.](http://www.jimmunol.org/10.4049/jimmunol.1300735)
tase 1 (41, 42). 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, FcγRIIB may also play context-dependent roles in IC-mediated inflammation. Whereas low expression of FcγRIIB is associated with SLE (44), high FcγRIIB 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 FcγRIIA and FcγRIIB versus FcγRIIA alone, which contrasts with the greater proteinuria following anti-

![Figure 5](http://www.jimmunol.org/Downloadedfrom/Mac-1-DELETION-ENABLES-HUMAN-LUPUS-SERA-INDUCED-NEPHRITIS)
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.
features similar to human disease include mesangial hypercellularity and mesangial deposits (mainly IgG), C3 deposition, endocapillary and extracapillary proliferation (crenests), 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 homologous human IgG, as normal human serum, some SLE-patient serum, and heat-aggregated human IgG failed to induce neutrophils 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.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

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References


Supplemental Information

Supplemental Figure 1.
A) Kinetics of albuminuria following transfer of SLE sera. Urinary albumin/creatinine ratio was evaluated after transfer of human SLE serum into IIA⁺IIIIB⁺γ−/− and IIA⁺IIIIB⁺γ−/−Mac-1−/− mice. Each data point represents one animal, the line indicates the median. *p<0.05, **p<0.01. B) Surface levels of FcγRIIA and FcγRIIIB and Mac-1 were evaluated in peripheral blood (Gr1⁺ cells), from IIA⁺IIIIB⁺γ−/− and IIA⁺IIIIB⁺γ−/−Mac-1−/− mice by flow cytometry using respective antibodies. The average of the MFI ± SEM was graphed. C) Analysis of circulating mouse anti-human IgG in indicated mice after SLE sera transfer. Circulating mouse anti-human IgG antibodies were measured by ELISA on serum collected from mice at day 14, using human IgG (100μg/mL) to coat the plate. D) Evaluation of proteinuria at day 14 of IIA⁺IIIIB⁺γ−/−Mac-1−/− mice that received sera from healthy donors (Normal, data pooled from 3 donors) or 5 SLE patients (1-5) (from Instituto Nacional de Ciencias Medicas y Nutricion, Salvador Zubiran, Mexico). Each data point represents one mouse, and the line indicates the median. *p<0.05, **p<0.01 compared to mice given normal serum.

Supplemental Figure 2.
A) IIA⁺γ−/−Mac-1−/− bone marrow confers susceptibility for the development of SLE-serum induced nephritis. Bone marrow from IIA⁺γ−/− and IIA⁺γ−/−Mac-1−/− mice were transferred into lethally irradiated γ−/−Mac-1−/− or γ−/− mice, respectively (or IIA⁺γ−/−Mac-1−/− into γ−/−Mac-1−/− as positive control). Three months later, mice received SLE serum intravenously. Left panel: proteinuria at day 14 after SLE serum injection is shown. Right panel: anti-CD11b (BD Pharmingen) immunohistochemistry following SLE-serum injection shows that kidneys are infiltrated by macrophages from the donor bone marrow. B) Immunohistochemistry with anti-F4/80 of spleen and kidney samples taken at day 14 after SLE serum injection from mice that were treated with liposomes containing PBS or clodronate. Macrophage infiltration observed in spleen and kidney (arrow) of PBS liposome treated mice is reduced in clodronate liposome treated animals. C) FcγRIIA and LFA-1 expression in Gr1⁺ neutrophils in the peripheral blood and the cremaster after RPA induction. 3hrs after RPA induction, blood was collected from the retroorbital plexus and the cremaster was digested (Collagenase type I and DNAse) to obtain a single cell suspension. Samples were analyzed by flow cytometry using anti-FcγRIIA and anti-LFA
antibody. Average of 3 mice per group ±SEM is shown. Dashed line represent average of MFI of isotype control antibodies.

Supplemental Tables S1.

A) Immune complex and IgG levels in human SLE sera. Immune complexes (ICs) and total human IgG were quantified by ELISA in indicated sera. “+” refers to its capacity to deposit in glomeruli and induce nephritis; thus the designation “pathogenic” sera. ND= not detectable.

B) Class of lupus nephritis and autoantibody profile of SLE patients in the study.

C) Peripheral blood analysis in mice. Blood collected at day 21 after SLE sera “A” injection, or from untreated mice was analyzed using an automated cell counter (Hemavet). No significant differences (p-value, ns) were observed between IIA+IIB+γ/⁻ and IIA+IIIB+γ/⁻Mac-1⁻ mice in untreated or SLE serum treated groups.
### A. Immune Complex and IgG levels in human SLE sera

<table>
<thead>
<tr>
<th>Source</th>
<th>Designation (+ = IgG-IC deposition in kidney)</th>
<th>ICs (µg Eq/mL)</th>
<th>hlgG (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control donor</td>
<td>-</td>
<td>ND</td>
<td>16.3</td>
</tr>
<tr>
<td>Patient SLE-A</td>
<td>+</td>
<td>5.2</td>
<td>10.5</td>
</tr>
<tr>
<td>Patient SLE-B</td>
<td>+</td>
<td>11.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Patient SLE-C</td>
<td>+</td>
<td>18.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Patient SLE-E</td>
<td>+</td>
<td>5.1</td>
<td>Not determined</td>
</tr>
<tr>
<td>Patient SLE-D</td>
<td>-</td>
<td>5.8</td>
<td>14.4</td>
</tr>
<tr>
<td>Patient SLE-F</td>
<td>-</td>
<td>7.3</td>
<td>12.8</td>
</tr>
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</table>

### B. Class of lupus nephritis and autoantibody profile of SLE patients in the study

<table>
<thead>
<tr>
<th>SLE-Serum</th>
<th>Pathogenic nephritis</th>
<th>Lupus nephritis</th>
<th>Autoantibodies</th>
<th>Low complement</th>
<th>SLEDA(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>Class IIA</td>
<td>ANA +</td>
<td>+</td>
<td>0-20</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>Unknown</td>
<td>RNP +</td>
<td>+</td>
<td>0-36</td>
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<tr>
<td>C</td>
<td>+</td>
<td>Class IV</td>
<td>Sm +</td>
<td>+</td>
<td>12-16</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>Class IV</td>
<td>SSA -</td>
<td>+</td>
<td>0-12</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>-</td>
<td>SSB +</td>
<td>+</td>
<td>5-10</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>Class IV</td>
<td>dsDNA +</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>


\(^b\) Serum samples were obtained from patients at several time points during different periods of disease activity. The pathogenic capacity of the sera was not modified by variations in the autoantibody levels (including dsDNA levels), overall SLE disease activity index (SLEDAI) and renal disease activity (as defined by proteinuria, hematuria and pyuria) at the time of the blood draw.

### C. Peripheral blood analysis in mice

<table>
<thead>
<tr>
<th></th>
<th>Untreated (n=3)</th>
<th>SLE serum (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II A(^{+})II B(^{+})γ(^{+})</td>
<td>II A(^{+})II B(^{+})γ(^{+})Mac-1(^{+})</td>
<td>II A(^{+})II B(^{+})γ(^{+})</td>
</tr>
<tr>
<td>Total Leukocytes (K/µL)</td>
<td>2.89 ± 0.40</td>
<td>4.99 ± 0.81</td>
<td>4.55 ± 0.83</td>
</tr>
<tr>
<td>Neutrophils (K/µL)</td>
<td>0.67 ± 0.14</td>
<td>2.42 ± 0.43</td>
<td>1.70 ± 0.34</td>
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<tr>
<td>Lymphocytes (K/µL)</td>
<td>1.29 ± 0.23</td>
<td>2.23 ± 0.44</td>
<td>2.59 ± 0.54</td>
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<tr>
<td>Monocytes (K/µL)</td>
<td>0.18 ± 0.01</td>
<td>0.24 ± 0.09</td>
<td>0.21 ± 0.54</td>
</tr>
<tr>
<td>Red Blood Cells (M/µL)</td>
<td>4.62 ± 0.93</td>
<td>7.59 ± 0.73</td>
<td>6.47 ± 0.86</td>
</tr>
<tr>
<td>Platelets (K/µL)</td>
<td>706.33 ± 138.80</td>
<td>1156.33 ± 191.17</td>
<td>1298.63 ± 182.95</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>5.33 ± 1.10</td>
<td>7.61 ± 0.60</td>
<td>6.60 ± 0.75</td>
</tr>
</tbody>
</table>