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Aberrant Macrophages Mediate Defective Kidney Repair That Triggers Nephritis in Lupus-Susceptible Mice

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CSF-1, required for macrophage (Mø) survival, proliferation, and activation, is upregulated in the tubular epithelial cells (TECs) during kidney inflammation. CSF-1 mediates Mø-dependent destruction in lupus-susceptible mice with nephritis and, paradoxically, Mø-dependent renal repair in lupus-resistant mice after transient ischemia/reperfusion injury (I/R). We now report that I/R leads to defective renal repair, nonresolving inflammation, and, in turn, early-onset lupus nephritis in preclinical MRL/MpJ-Fas lpr/Fas lpr mice. Moreover, defective renal repair is not unique to MRL-Fas lpr mouse, as flawed healing is a feature of other lupus-susceptible mice (Sle 123) and MRL mice without the Fas gene mutation. Increasing CSF-1 hastens renal healing after I/R in lupus-resistant mice but hinders healing, exacerbates nonresolving inflammation, and triggers more severe early-onset lupus nephritis in MRL-Fas lpr mice. Probing further, the time-related balance of M1 “destroyer” Mø shifts toward the M2 “healer” phenotype in lupus-resistant mice after I/R, but M1 Mø continue to dominate in MRL-Fas lpr mice. Moreover, hypoxic TECs release mediators, including CSF-1, that are responsible for stimulating the expansion of M1 Mø inherently poised to destroy the kidney in MRL-Fas lpr mice. In conclusion, I/R induces CSF-1 in injured TECs that expands aberrant Mø (M1 phenotype), mediating defective renal repair and nonresolving inflammation, and thereby hastens the onset of lupus nephritis. The Journal of Immunology, 2012, 188: 000–000.

Identifying molecular mechanisms that mediate experimental lupus nephritis offer the promise of uncovering novel therapeutic targets for human lupus. MRL/MpJ-Fas lpr/Fas lpr mice (MRL-Fas lpr mice) are powerful tools for dissecting mechanisms central to lupus nephritis and are a multiorgan (kidney, skin, lung, salivary/lacrimal glands, and so on) disease animal model similar to that of human lupus (1–3). As in human lupus, kidney disease in MRL-Fas lpr mice is the major cause of mortality. Macrophages (Mø) are prominent within the inflamed kidneys (4, 5) and are key mediators of lupus nephritis in MRL-Fas lpr mice (6–10). Thus, Mø are prime candidates as key regulators of lupus nephritis.

CSF-1, required for Mø survival, differentiation, and proliferation, incites inflammation that leads to Mø-mediated destruction. We established that CSF-1 and Mø are pivotal in the pathogenesis of lupus nephritis in MRL-Fas lpr mice based on the following evidence. CSF-1 is detected in tubular epithelial cells (TECs) that are surrounded by Mø at the onset of lupus nephritis (4, 10). Mice deficient in CSF-1 (Csf1−/−; MRL-Fas lpr), nearly depleted of Mø, are protected from lupus nephritis (11). Local, discrete intrarenal CSF-1 overexpression incites Mø-rich inflammation in the adjacent tissue (12), and systemic CSF-1 overexpression hastens the onset and progression of Mø-rich lupus nephritis (10). Probing further, we see that CSF-1 is upregulated largely by TECs at the onset of renal inflammation and spills over into the circulation, thereby increasing the number of circulating monocytes and shifting their phenotype toward an “inflammatory,” activated population (10). These inflammatory Mø are more readily recruited to the kidney (10) and release mediators that induce apoptosis of renal parenchymal cells, thereby damaging the kidney (10, 13). Moreover, CSF-1 and Mø are highly relevant to human lupus nephritis. Enhanced intrarenal CSF-1 and Mø correlate with a higher histopathology activity index, and heightened serum and urine CSF-1 levels correlate with increased disease activity (10). Taken together, CSF-1 and Mø are closely intertwined in mediating lupus nephritis.

Paradoxically, CSF-1 and Mø mediate renal repair in lupus-resistant mice. As inflammation during a transient insult is meant to set the stage for repair, and Mø are implicated in tissue repair (14, 15), we selected a model of transient renal injury leading to repair, ischemia/reperfusion injury (I/R), and determined that CSF-1 promotes Mø-dependent renal repair. Tubular damage is a cardinal feature of ischemic renal injury and is reversible as uninjured TECs proliferate to replenish faulty TECs.
Injecting CSF-1 after I/R hastened healing (decreased tubular disease and fibrosis, improved renal function) in lupus-resistant mice (17). Notably, CSF-1 increased TEC proliferation and reduced further damage by suppressing TEC apoptosis. We determined that Mø contributed to renal repair, as eliminating Mø increased tubular abnormalities and renal fibrosis, suppressed TEC proliferation, and increased TEC apoptosis (17). Taken together, Mø and CSF-1 are intertwined and central to tubular repair in lupus-resistant mice.

How do CSF-1 and Mø mediate renal repair in lupus-resistant mice and, conversely, injury in lupus-susceptible mice? Mø regulate a dynamic balance between destructive and repair signals in the kidney. These mononuclear phagocytes are broadly and simplistically divided into a operationally useful conceptual framework that describes Mø along a continuum of functional state subsets referred to as classically activated “destroyers” (M1) and alternatively activated “healers” (M2) (14, 18–20). The M1 Mø release molecules that initiate tissue injury (21) evident in the kidney after I/R (20), whereas the M2 Mø generate molecules, such as TGF-β, that suppress further Mø activation and promote repair (22). Moreover, evidence suggests that phagocytosis of tissue debris may switch Mø from proinflammatory to anti-inflammatory (23, 24). However, phagocytosis is impared in Mø derived from lupus-susceptible mice, and these Mø are not able to efficiently clear apoptotic TECs (25) and other debris (G. Tesch and V. Kelley, unpublished observations). Thus, the dominance of M1 Mø may fail to switch to M2 Mø in lupus-susceptible mice. Moreover, inefficient engulfment of apoptotic cells may activate the immune system and trigger lupus (26).

The goal of this study was to uncover mechanisms responsible for CSF-1 leading to Mø-mediated kidney destruction in lupus-susceptible mice and Mø-mediated kidney repair in lupus-resistant mice. We used a transient insult (I/R) to unmask the molecular mechanisms that mediate defective innate immunity bridging adaptive immunity in lupus-susceptible mice. We now report that transient injury leads to defective repair, nonresolving inflammation, and early-onset lupus nephritis as aberrant Mø fail to switch from phenotypes that “destroy” (M1) to phenotypes that “heal” (M2) in lupus-susceptible MRL-Faslpr mice.

Materials and Methods

Mice
We purchased C57BL/6 (B6), BALB/c, MRL-Faslpr, MRL/MpJ (MRL++), and B6.Sle1Sle2Sle3Sle5 (Sle 123) mice from The Jackson Laboratory (Bar Harbor, ME). Mice were housed and bred at Harvard Medical School. The CSF-1−/−, MRL-Faslpr, and TgC/−/−MRL-Faslpr mice were constructed as previously detailed (10). Use of mice in this study was reviewed and approved by the Standing Committee on Animals in the Harvard Medical School, in adherence to standards set in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 86-23, revised 1996).

Ischemia/reperfusion injury
We anesthetized mice and exposed the left kidney through a flank incision. We induced ischemia by clamping the renal pedicle with nontraumatic microaneurysm clamps (Roboz, Gaithersburg, MD). We removed the clamps after 30 min in males, 45 min in females. The body temperature was controlled at 36.6–37.5°C throughout the procedure. We removed and prepared the kidneys as previously described (17, 27). We initiated I/R at: 6 wk of age in MRL-Faslpr B6, BALB/c, TgC+/−MRL-Faslpr, and CSF-1−/−; MRL-Faslpr mice; 6 wk and 8–10 mo of age in MRL++ mice; and 10 wk of age in Sle 123 mice.

Renal histopathology
We fixed kidneys in 10% neutral buffered formalin, embedded them in paraffin, and stained paraffin sections with periodic acid–Schiff reagent. Kidney disease was assessed as previously detailed (17).

Collagen detection
We stained paraffin sections after rehydration in picrosirius red solution for 1 h and rinsed (×2) with acidic water. We dehydrated and mounted the sections and analyzed the amount of stain using a Nikon Eclipse E1000 upright fluorescence microscope and Adobe Photoshop CS4 extended.

Renal function
We measured albuminuria as previously described (17).

Immunohistochemistry
We stained frozen kidney sections, fixed in 25% ethanol/75% acetone for 10 min at room temperature, for the presence of Mø, neutrophils, and T cell populations, using anti-mouse F4/80 Ab (BM-8; Invitrogen, Carlsbad, CA), anti-mouse GR-1 Ab (RB6-8C5; BD Pharmingen, San Diego, CA), anti-mouse CD4 Ab (RM4-5; eBioscience, San Diego, CA), anti-mouse CD8 Ab (53-6; 7e; eBioscience) (10). Optimal concentrations of primary Abs were diluted in Ab dilution buffer and incubated with the tissue sections overnight in a humidified chamber at 4°C. We incubated tissue sections with biotinylated anti-rat IgG Ab (BA-4001); Vector Laboratory, Burlingame, CA) for 1 h at room temperature, followed by incubation with ABC complex (PK-6100; Vector Laboratories) for 1 h at room temperature. Then, the stain was developed using diaminobenzidine peroxidase substrate (SK-4100; Vector Laboratories), followed by counterstain with Mayer’s Hematoxylin (Sigma-Aldrich, St. Louis, MO).

To determine the number of M1 and M2 Mø, we fixed frozen kidney sections in 4% paraformaldehyde [for inducible NO synthase (iNOS) staining] and washed nonfixed sections for 10 min in PBS (for CD206), then stained them with anti-mouse F4/80 Ab (Invitrogen), followed by Alexa Fluor 594 goat anti-rat IgG Ab (Invitrogen). Next, we stained them with FITC-conjugated rat anti-mouse CD206 (M5D3; Serotec, Raleigh, NC). For the staining of iNOS, the kidney sections were permeabilized in saponin buffer, then stained with FITC-conjugated anti-mouse iNOS Ab (BD Pharmingen). We enumerated the number of F4/80+ iNOS+ cells and F4/80+ CD206+ cells in 10 high-power fields and divided the number of F4/80+ iNOS+ cells by the number of F4/80+ iNOS− cells for the M1/M2 ratio.

Kidney injury molecule 1 expression
Cryosections were washed in PBS for 10 min and stained with polyclonal anti-kidney injury molecule 1 (Kim-1) (anti-sera R9) to identify tubular injury, as previously detailed (17). Real-time PCR was performed as described before (17). Kim-1 primers (Invitrogen; sense, 5′-GGAGGAGTGGGGGGGG-3′; antisense, 5′-AGGACAGAATGGGATTGC-3′) were used, and mRNA levels were normalized to GAPDH, 5′-CATGCCCTCACAGTGAAG-3′; antisense, 5′-CCTAGGCCCCCTCTCTTTAAT-3′.

IgG and C3 deposits within renal glomeruli
Cryosections were fixed in 20% acetone/80% methanol for 15 min at −20°C, then stained with FITC-conjugated goat anti-mouse IgG and FITC-conjugated goat IgG fraction of mouse C3 (Cappel, Malvern, PA) (10). The fluorescence intensity within the peripheral glomerular capillary walls and the mesangium was scored on a scale of 0–3 (0 = none; 1 = weak; 2 = moderate; 3 = strong). At least 10 glomeruli per section were analyzed.

Flow cytometry
We prepared and stained single-cell suspensions from kidneys or primary cultured TEC/bone marrow (BM) Mø as described previously (17). To identify intracellular cytokine expression, kidneys or cultured cells were incubated in RPMI 1640 containing Monensin (Sigma-Aldrich) for 4 h. We washed the cells in PBS; suspended them in FACS buffer (PBS, 5% FBS, and 0.09% Na2S), and incubated them with several cell surface markers for 30 min on ice. Then we fixed and permeabilized the cells, using Fix/Perm buffer (BioLegend, San Diego, CA). After staining of intracellular cytokines, cells were washed twice in Fix/Perm buffer and suspended in FACS buffer to allow the rescaling of permeabilized membrane. We collected 1.0 to 5.0 × 105 total kidney cells and 1.0 to 3.0 × 104 cultured cells using the FACSCalibur (Becton Dickinson, San Jose, CA) and analyzed data using FlowJo software 9.3 (Tree Star, Palo Alto, CA).

Abs
We used the following Abs for FACS analysis: FITC-conjugated anti-CD4 (L3T4; eBioscience), anti-CD8 (53-6; 7e; eBioscience), anti-CD3 (145-2C11), anti-B220 (RA3-6B2; eBioscience), anti-CD206 (MCA2235PA;
Serotech), anti–TNF-α (MP6-XT22; BD Pharmingen), anti–CD80 (16-10A1; BD Pharmingen), anti–CD11b (M1/70; BD Pharmingen), anti–CD45.1 (A20; BD Pharmingen) and anti–CD45.2 (104; eBioscience), PE-conjugated anti–IL-10 (JES5-16E3; eBioscience), anti–IL-4R (mIL4R-M1; BD Pharmingen), anti–GR-1 (RB6-8C5; BD Pharmingen), anti–Ly6G (1A8; BD Pharmingen), anti–CD86 (GL1; eBioscience), anti–CD45.1 (A20) and anti–CD45.2 (104) PerCP-Cy5.5 conjugated anti–CD11b (M1/70; BD Pharmingen) and allophycocyanin-conjugated anti–F4/80 (BMS; BioLegend), anti–CD45.1 (A20), and anti–CD45.2 (104). We used biotin conjugated anti–Ly6C (AL-21; BD Pharmingen), followed by Streptavidin-PerCP-Cy5.5 (BD Pharmingen).

**Serum Ig profile**

We measured total IgG and total anti-dsDNA Abs, as previously reported.(3).

**Mo cell culture**

We isolated and cultured BM Møs, as previously described, from mice 6–8 wk of age (5). BM cells were cultured in L929 cell-conditioned medium to separate adherent differentiated cells. Nonadherent, immature cells were removed and cultured for 3–4 d, as previously detailed (10).

**C1 cell culture**

The immortalized mouse proximal TEC cell line (C1) (28) was grown to be subconfluent on collagen-coated plates in modified K1 media, as previously explained (17).

**Ab/cytokine administration**

We injected mice (i.p.) every 12 h with purified human rCSF-1 (gift from Chiron, Emeryville, CA), using a dose of 50 μg/kg body weight beginning at 1.5 d and ending at 5 d (17) after I/R. Mice injected with PBS were used as controls.

**Mo stimulation using supernatant from hypoxic TEC (C1)**

C1 cells were exposed to 5% CO2, balance N2 (3% O2), for 24 h in a hypoxic chamber with 1% FCS media. Supernatant was collected after 24 h (day 1). Reoxygenation was performed by placing the cells in an incubator gassed with 95% air/5% CO2 with C1 cultured media. Then supernatant was changed to 1% FCS media at 4 d and collected at 5d. Møs were incubated with C1 supernatant for 24 h before Mo profiling.

**Cell survival/proliferation**

We analyzed proliferation using the MTT colorimetric assay (Roche, Palo Alto, CA) according to the manufacturer’s instructions.

**Real-time PCR**

Real-time PCR was performed as described before (17). We detected TNF-α, iNOS, arginase-1, IL-10, and GAPDH expression, using QuantTect Primer Assays (Qiagen, Valencia, CA). The data were analyzed by the ∆∆Ct method.

**Statistics**

Data represent the mean ± SEM prepared using GraphPad Prism software, version 4.0. We used the nonparametric Mann–Whitney U test to evaluate p values. A value of p < 0.05 was considered significant.

**Results**

**Renal repair is defect in lupus-susceptible MRL-Fas<sup>lpr</sup> mice after transient kidney injury (I/R)**

Our prior studies show that CSF-1 hastens renal repair after transient renal injury (I/R) and is expressed by TECs in normal (lupus-resistant) mice (17). In contrast, CSF-1 hastens the tempo of lupus nephritis in MRL-Fas<sup>lpr</sup> mice (10). Thus, we tested the hypothesis that transient renal injury (unilateral I/R) leads to unresolved inflammation and defective repair in MRL-Fas<sup>lpr</sup> mice. To test this hypothesis, we compared the time-related magnitude of renal injury and repair in preclinical MRL-Fas<sup>lpr</sup> mice and lupus-resistant, C57BL/6 (B6) and BALB/c, mice. We detected a similar initial loss of renal function (albuminuria), an index of renal injury and repair, that peaks at 3 d after I/R in lupus-resistant and MRL-Fas<sup>lpr</sup> mice and returns to normal (baseline) levels in lupus-resistant (20 d), but not MRL-Fas<sup>lpr</sup>, mice (Fig. 1A). This finding suggests that renal repair is defective in MRL-Fas<sup>lpr</sup> mice. As tubular injury in the outer medulla of the kidney is exquisitely sensitive to hypoxic injury after I/R, we evaluated tubular disease and Kim-1, an index of tubule injury (29), in MRL-Fas<sup>lpr</sup> and lupus-resistant mice. The magnitude of tubular abnormality (casts, dilated tubules) in the outer medulla reached an apex at 3 d after I/R in lupus-resistant and MRL-Fas<sup>lpr</sup> mice, declined, and returned to baseline at 20 d after I/R in lupus-resistant mice. In contrast, tubular disease only partially resolved in MRL-Fas<sup>lpr</sup> mice even by 40 d after I/R (Fig. 1B1). Defective renal repair in the less severely impacted renal cortex was similar to that in the outer medulla in MRL-Fas<sup>lpr</sup> (data not shown). Consistent with renal tubule disease, we found maximal Kim-1 expression in lupus-resistant and MRL-Fas<sup>lpr</sup> mice at 3 d after I/R that declined and returned to baseline by 20 d in lupus-resistant mice, but remained elevated in MRL-Fas<sup>lpr</sup> mice (Fig. 1B2). Strikingly, leukocyte infiltrates, an index of inflammation that accompanies tubular injury, descended rapidly in lupus-resistant mice but continued to ascend in MRL-Fas<sup>lpr</sup> mice. This finding suggests that infiltrating leukocytes may be central to nonresolving inflammation and may mediate further renal destruction (Fig. 1C1). In keeping with increasing renal injury, rather than repair, in MRL-Fas<sup>lpr</sup> mice, the time-related magnitude of fibrosis (Sirius red) increased more dramatically in MRL-Fas<sup>lpr</sup> than in lupus-resistant mice (Fig. 1C2). As expected, the contra-lateral kidneys in MRL-Fas<sup>lpr</sup> and lupus-resistant mice resemble age-matched sham controls (data not shown). Of note, male mice are more sensitive than female mice to ischemic injury; thus, we initially used MRL-Fas<sup>lpr</sup> male mice (30). By extending the ischemic injury (from 30 min to 45 min) in female mice, we detect renal injury and repair comparable to that in male lupus-resistant mice. Using this protocol, we found that the magnitude of defective repair after I/R in age-matched MRL-Fas<sup>lpr</sup> female mice mimicked that in males (data not shown). Moreover, overly aggressive inflammation at the onset of injury is not responsible for defective renal repair in MRL-Fas<sup>lpr</sup> mice after I/R. We detected a similar magnitude of time-related tubular abnormality (0, 2, 12, 24, 72 h) in the outer medulla and cortex, Kim-1 expression (0, 24, 48, 72 h), expression of renal function (72 h), and infiltrating leukocytes (Mo [F4/80]), T cells [CD4, CD8, and CD3<sup>+</sup> B220<sup>+</sup> CD4<sup>+</sup> CD8<sup>+</sup>], B cells [B220<sup>+</sup>], and granulocytes (GR1<sup>+</sup>) (72 h) in MRL-Fas<sup>lpr</sup> and lupus-resistant mice (data not shown). These findings taken together show that renal repair is defective in lupus-susceptible MRL-Fas<sup>lpr</sup> mice after transient renal injury (I/R).

**Renal I/R triggers early-onset lupus nephritis in MRL-Fas<sup>lpr</sup> mice**

As renal repair after transient injury (I/R) is defective in MRL-Fas<sup>lpr</sup> mice, we tested the hypothesis that transient renal injury leads to nonresolving inflammation triggering early-onset lupus nephritis. Lupus nephritis and renal I/R share several pathological features, including tubular abnormality, an influx of leukocytes into the interstitium, and the loss of renal function. However, glomerular disease, perivascular leukocyte infiltrates, and autoantibodies are features of lupus nephritis, not renal I/R, in MRL-Fas<sup>lpr</sup> mice. Therefore, to test our hypothesis we compared the time-related magnitude of features specific to lupus nephritis (glomerular disease, perivascular abnormality, and serum autoantibodies) in MRL-Fas<sup>lpr</sup> mice after I/R with controls (MRL-Fas<sup>lpr</sup> age-matched shams). We detected a greater time-related increase in glomerular disease in MRL-Fas<sup>lpr</sup> kidneys after I/R, compared with the contralateral kidney and control
kidneys (Fig. 2A1). Consistent with this finding, the time-related magnitude of IgG and C3 deposits in glomeruli was greater in MRL-Faslpr kidneys after I/R compared with the contralateral kidney and control kidneys (Fig. 2A2). Even more dramatic was the prominent time-related rise in the number of leukocyte infiltrates accumulating around a large proportion (incidence) of renal vasculature after I/R in MRL-Faslpr mice that was barely seen in age-matched MRL-Faslpr control mice compared with lupus-resistant controls (Fig. 2B). In addition, the time-related rise in serum autoantibodies (anti-dsDNA, IgG (IgG1, IgG2a, IgG2b, IgG3, not shown), and C3 was higher in MRL-Faslpr mice after I/R, compared with controls (Fig. 2C). Lupus-resistant, B6 (Fig. 2) and BALB/c (data not shown) kidneys with and without I/R were similar and served as negative controls. As transient injury to the kidney (I/R) is responsible for increasing circulating autoantibodies, this suggests that neo-antigens released from the

FIGURE 1. Defective renal repair in MRL-Faslpr mice compared with lupus-resistant mice after I/R injury. Male MRL-Faslpr and lupus-resistant (BALB/c and B6) mice were analyzed at increasing times (d) after I/R. We initiated I/R at 6 wk of age. (A) Renal function (albuminuria, μg/24 h). (B) Tubular histopathological features (dilated tubules, casts) in the outer medulla and Kim-1 expression (index of tubule injury). (C) Interstitial leukocyte infiltrates and fibrosis (collagen staining, Sirius red). Representative photomicrographs 40 d after I/R (original magnification ×40, except Kim-1, ×20). Data represent means ± SEM. Data are representative of three separate experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
injured kidney may be responsible for triggering the production of autoantibodies. Importantly, the contralateral kidneys remained similar to control kidneys; thus, elevating serum autoantibodies alone, without direct kidney injury, is not sufficient to initiate lupus nephritis (Fig. 2). All these data taken together indicate that transient ischemic kidney injury triggers nonresolving inflammation, thereby leading to a rise in circulating autoantibodies and early-onset lupus nephritis in the injured kidney.

Defective repair after I/R is not unique to MRL-Faslpr mice, but linked to lupus susceptibility and the MRL background

To determine if defective repair is a more global feature of lupus-susceptible mice, and not unique to MRL-Faslpr mice, we evaluated renal repair after I/R in another lupus-susceptible strain, Sle123, that develops lupus nephritis (31, 32). We compared renal injury and repair in preclinical Sle123, MRL-Faslpr mice (positive control), and lupus-resistant, B6 and BALB/c mice (negative controls) after I/R. Recall that renal injury is repaired by 20 d after I/R in lupus-resistant mice (Fig. 1). In contrast, renal repair is defective in Sle123 mice after I/R. The magnitude of tubule pathology (outer medulla) and tubule injury (Kim-1) is substantial in Sle123 mice compared with lupus-resistant mice after I/R (day 20) (Fig. 3A). Defective tubular repair in Sle123 mice in the cortex is similar to that in the outer medulla (data not shown). Moreover, the magnitude of interstitial disease, leukocyte infiltrates, and fibrosis, as well as loss of renal function (albuminuria), is elevated in Sle123 mice compared with lupus-resistant mice after I/R (20 d) (Fig. 3B). Of note, the extent of defective repair in Sle123 and MRL-Faslpr mice is comparable (Fig. 3). As in lupus-susceptible MRL-Faslpr mice, renal repair is defective in lupus-susceptible Sle123 mice after I/R.
To test the hypothesis that the MRL background, not the Faslpr mutation alone, is responsible for defective repair, we compared renal repair in MRL-++ mice with the Faslpr mutation (deficient in Fas) with renal repair in MRL-++ mice without the Faslpr mutation (Fas intact) after I/R. It is important to appreciate that lupus nephritis in MRL-++ mice is latent (clinically evident in the second year of life), progresses at a slower pace (50% mortality at 17 mo of age), and is less severe compared with the fulminant (clinically evident at 3–4 mo of age), lethal (50% mortality at 5 mo of age) renal disease in MRL-Faslpr mice. We evaluated pre-clinical MRL-++ mice at a point (20 d) after I/R when the kidneys in lupus-resistant mice are healed. Renal tubular injury does not fully resolve in MRL-++ mice at 1.5 mo of age after I/R, as the tubular disease, Kim-1 expression, and leukocyte infiltrates in the interstitium are substantially greater than in B6 and BALB/c mice (Fig. 3A, right panel). Because lupus nephritis is latent in mice without the Fas, we tested the hypothesis that defective repair after I/R is more pronounced in preclinical MRL-++ mice that are aged closer to the emergence of spontaneous kidney disease (33), a situation more comparable to that in MRL-Faslpr mice. The magnitude of

**FIGURE 3.** Defective renal repair after I/R is not unique to MRL-Faslpr mice, as it is a feature in other lupus-susceptible mice without the Faslpr mutation. Renal repair in Sle123 female mice (left panel) and MRL-++ male mice (right panel) after I/R (20 d) are displayed. We initiated I/R in Sle123 mice at 10 wk of age and MRL-++ mice at 1.5 and 8–10 mo of age. Age-matched lupus-resistant (BALB/c [female] and B6 [male]) mice after I/R served as a negative control, and MRL-Faslpr male mice after I/R served as a positive control. Note BALB/c and B6 males and females respond similarly. (A) Renal tubular histopathological features (dilated tubules, casts) in the outer medulla and Kim-1 expression. (B) Interstitial leukocyte infiltrates and fibrosis (Sirius red). (C) Renal function (albuminuria, μg/24 h). Dotted line denotes baseline values for age-matched mice that are not manipulated. Data represent means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
renal tubular (Fig. 3A) and interstitial (Fig. 3B) disease after I/R is greater in preclinical MRL-++ (8–10 mo of age) than in MRL-++ (1.5 mo of age), and is similar to that in MRL-Faslpr mice (1.5 mo of age) (Fig. 3, right panel). This finding is in keeping with CSF-1–producing cells implanted under the kidney capsule, initiating nonresolving renal inflammation in preclinical, similarly aged MRL-++ mice, but not in younger (1.5 mo of age) MRL-++ mice (A. Schwarting and V.R. Kelley, unpublished observations). In addition, defective renal repair is not a general feature of other autoimmune diseases, as the kidneys of preclinical NOD mice healed as rapidly as those of BALB/c and B6 mice after I/R (data not shown). Of note, serum CSF-1 is elevated in MRL-Faslpr mice after I/R, but does not rise in NOD mice after I/R (data not shown) (17). In sum, defective renal repair after I/R is not unique to MRL-Faslpr mice but is characteristic of other lupus-susceptible mice expressing Fas.

Does defective repair lead to early-onset lupus nephritis in these other lupus-susceptible strains? We detected more glomerular disease in the kidney of Sle 123 mice after I/R compared with the contralateral and age-matched control kidneys, and serum IgG levels were higher in Sle 123 mice after I/R than in age-matched controls (Supplemental Fig. 1, left panel). Lupus-resistant (B6 and BALB/c) mice served as negative controls. Moreover, glomerular and perivascular disease in the preclinical MRL-++ mice at 1.5 mo of age was greater than that in the contralateral control kidney, and this is even more pronounced in MRL-++ at 8–10 mo of age after I/R (Supplemental Fig. 1, right panel). In addition, the trend in serum IgG was higher in MRL-++ mice after I/R compared with controls. As the spontaneous time course of the disease is somewhat slower in Sle 123 (50% mortality at 8 mo of age) (31) and dramatically slower in MRL-++ mice (50% mortality at 17 mo of age) (33, 34) and lupus nephritis in both strains is substantially more heterogeneous than in MRL-Faslpr mice, it is not surprising that early-onset lupus-nephritis is somewhat less striking in these strains compared with MRL-Faslpr mice.

CSF-1 mediates defective repair, not healing, in MRL-Faslpr mice

CSF-1 mediates healing in lupus-resistant mice (17). In contrast, CSF-1 mediates destructive inflammation and lupus nephritis in MRL-Faslpr mice (10). Therefore, we tested the hypothesis that increasing CSF-1 in MRL-Faslpr mice heightens the severity of defective repair and nonresolving inflammation leading to early-onset lupus nephritis after I/R. To test this hypothesis, we used mutant MRL-Faslpr mice that we constructed to express differing levels of CSF-1 in the kidney and circulation: TgC/+;MRL-Faslpr (high), MRL-Faslpr (wildtype [WT], moderate), and CSF-1op/op;MRL-Faslpr (none), as previously detailed (10). We compared renal injury and repair in these MRL-Faslpr mice with differing levels of CSF-1 at a time point when lupus-resistant kidneys are repaired (20 d) after I/R. We detected the greatest magnitude of tubular disease and injury (Kim-1), interstitial infiltrating leukocytes, and fibrosis in MRL-Faslpr mice expressing the highest level of CSF-1 (TgC/+;MRL-Faslpr > WT > CSF-1op/op;MRL-Faslpr) (Fig. 4). Of note, MRL-Faslpr mice lacking CSF-1 are spared from renal disease after I/R (20 d). Consistent with this finding, injecting CSF-1 after I/R, using the same protocol that promotes healing in lupus-resistant mice (17), thwarts healing in MRL-Faslpr mice (data not shown). Moreover, specific characteristics of lupus nephritis that are not features of I/R, including glomerular disease (Fig. 4C) and immune deposits (IgG, C3) lodged in glomerular capillary walls (data not shown), perivascular infiltrates, serum autoantibodies (IgG, dsDNA), and the loss of renal function (Fig. 4C) are most pronounced in MRL-Faslpr mice expressing the highest level of CSF-1 (TgC/+;MRL-Faslpr > WT > CSF-1op/op;MRL-Faslpr). Note that the contralateral kidneys for TgC/+;MRL-Faslpr, WT, and CSF-1op/op;MRL-Faslpr mice did not have features of I/R or lupus nephritis. It appears that increasing CSF-1 in the kidney and circulation diminishes renal repair and, in turn, promotes more severe early-onset lupus nephritis in MRL-Faslpr mice after I/R.

MRL-Faslpr Mø in the kidney are skewed toward an M1 (destroyer), rather than M2 (healer), phenotype after I/R and during spontaneous lupus nephritis

Mø are integral in inflammation leading to repair and, in contrast, inflammation leading to injury. The initial (3, 5 d) increase in intrarenal Mø (F4/80+, CD68+) is similar in lupus-resistant and MRL-Faslpr mice after I/R. However, Mø dramatically and continuously decline (10, 20, 40 d) in lupus-resistant mice, whereas Mø remain elevated (10, 20 d) and then begin to reascend (40 d) in MRL-Faslpr mice (Fig. 5A). Of note, we did not detect a differential expression of neutrophils in MRL-Faslpr and B6 mice after I/R, as neutrophils (Ly6G+, F4/80+) peaked rapidly (day 1) and similarly declined promptly, returning to baseline levels (day 20) in MRL-Faslpr and B6 mice (Supplemental Fig. 2B). As inflammation is nonresolving and leads to lupus nephritis in MRL-Faslpr mice and, in contrast, to healing in lupus-resistant mice, this suggests that Mø, not neutrophils, in MRL-Faslpr are central to defective repair and escalating destructive inflammation.

Simplistically, Mø phenotypes are divided into classically activated Mø that are central to mediating tissue destruction, termed M1, and alternatively activated Mø that are central to repair, termed M2. We tested the hypothesis that Mø shift from M1-like “destroyers” to M2-like “healers” in lupus-resistant mice, whereas M1 Mø continue to rise and fail to shift toward the M2 phenotype in MRL-Faslpr mice, after I/R. To phenotype Mø populations by flow cytometry, we identified the CD45+ hematopoietic cells and, within this population, the CD11b+ cells. As F4/80hi reflect the M1 phenotype and F4/80lo largely reflect the M2 phenotype (35), we further analyzed CD11b+ leukocytes into F4/80hi and F4/80lo Mø populations (Fig. 5B1). Moreover, we compared the time-related magnitude of CD11b+ F4/80hi and F4/80lo Mø in the kidney, using specific panels of M1 (iNOS, TNF-α, CD86, IL-12) and M2 (CD206, IL-10, Ym-1) markers in lupus-resistant and MRL-Faslpr mice after I/R. We arbitrarily divided the phases after I/R into initial phase (0–3 d), initiation of injury in lupus-resistant mice, and later phase (5–20 d), progression toward repair in lupus-resistant mice. We detected a dramatic and similar rise in M1 (F4/80lo) Mø in lupus-resistant and MRL-Faslpr mice during the initial phase after I/R (Fig. 5B2, 5B3), a finding that is similar using M1-specific markers (Fig. 5B4). However, M1 Mø (number, Fig. 5B3, 5B4; frequency and representative plots, Supplemental Fig. 3) markedly declined and returned to normal levels in lupus-resistant mice but remain elevated in MRL-Faslpr mice. Moreover, we identified CD11b+ F4/80hi GR1hi that peaked at 5 d after I/R in MRL-Faslpr and B6 kidneys and that declined in B6 mice, but remained elevated in MRL-Faslpr mice, at 20 d after I/R (Supplemental Fig. 2C). By comparison, during the initial phase after I/R, the magnitude of M2 Mø barely increases in lupus-resistant and MRL-Faslpr mice (Fig. 5B3, 5B4). Moreover, we noted a far more robust rise in M2 Mø in lupus-resistant mice compared with MRL-Faslpr mice at the beginning of the later phase after I/R (day 5) that declines to near baseline levels (day 20, day 40) (Fig. 5B3, 5B4). In addition, the failure to shift from M1 to M2 and nonresolving inflammation characterize Sle 123 after I/R, and thus are not features unique to MRL-Faslpr mice (Fig. 6). However, as the accumulation of Mø in MRL-Faslpr kidneys is nearly 2-fold...
greater than in Sle 123 kidneys after I/R (data not shown), lupus nephritis in MRL-Faslpr mice may be more dependent on Mø-mediated mechanisms than are Sle 123 mice (Fig. 6). Taken together, the balance of M1 and M2 Mø shifts from the M1 phenotype, at the onset of injury, to the M2 phenotype, during healing after I/R in lupus-resistant mice, whereas M1 Mø continue to dominate in MRL-Faslpr mice after I/R during defective repair and nonresolving inflammation that triggers early-onset lupus nephritis. We wish to point out that most, but not all, Mø express M1 or M2 markers (immunostaining, flow cytometry, Supplemental Fig. 4), suggesting that other Mø populations are yet to be clearly defined in the inflamed kidney.

Is there a shift toward M1 Mø rather than M2 Mø during spontaneous lupus nephritis in MRL-Faslpr mice? We compared the Mø M1 and M2 phenotypes in the kidney prior to (6 wk of age) and during advanced (5 mo of age) lupus nephritis in MRL-Faslpr mice. Moreover, these Mø M1 and M2 phenotypes were compared with MRL-Faslpr mice after I/R (day 20). The number of M1 (F4/80low and M1 markers) and M2 (F4/80hi and M2 markers) Mø increase in spontaneous lupus nephritis; however, the rise in the number of M1 Mø is far more robust than that in M2 Mø, and even exceeds the number of M1 Mø in the kidney after I/R (Fig. 7). Thus, a greater abundance of M1 than of Mø is present in the kidney during spontaneous lupus nephritis. We wish to re-emphasize that Mø are not crisply divided into M1 and M2 categories, but rather that we use this conceptual framework to describe a continuum of Mø along a spectrum of leukocytes with diverse functions.

Hypoxic cultured TECs release mediators that skew Mø toward the M1 phenotype in MRL-Faslpr and lupus-resistant mice during injury and shifts Mø toward the M2 phenotype in lupus-resistant, but not MRL-Faslpr mice, during repair. During injury, TECs release mediators, including CSF-1, responsible for mediating repair in lupus-resistant mice (17) and lupus nephritis in MRL-Faslpr mice (10). As shown, in the later
phase after I/R, intrarenal Mø are skewed toward the M1 phenotype in MRL-Faslpr mice when destructive inflammation is escalating and toward the M2 phenotype in lupus-resistant mice when the kidney is healing (Fig. 5). As hypoxia induces TEC injury, we tested the hypothesis that hypoxic tubules release mediators that skew Mø toward the M1 phenotype in MRL-Faslpr mice and toward the M2 phenotype in lupus-resistant mice. To test this hypothesis, we induced hypoxia in a proximal TEC line (C1) that we previously constructed (28). We then stimulated BM Mø from preclinical MRL-Faslpr and lupus-resistant mice with supernatant taken from hypoxic tubules just after injury (1 d) and during proliferative repair (5 d), as verified by Kim-1 expression and survival/proliferation (MTT assay) (data not shown). Mediators, including CSF-1, released from hypoxic cells during injury (1 d) stimulated a greater magnitude of M1 BM Mø (TNF-α, CD86, iNOS, flow cytometry, and/or real-time PCR) than M2 BM Mø (IL-10, CD206, arginase-1, flow cytometry, real-time PCR) in MRL-Faslpr mice compared with lupus-resistant mice. However, the magnitude of M1 Mø was greater in MRL-Faslpr than lupus-resistant mice (Fig. 8). In contrast, hypoxic TECs released mediators during repair that stimulated more M2 BM Mø (IL-10, CD206, arginase-1, flow cytometry, and/or real-time PCR) in lupus-resistant than in MRL-Faslpr mice (Fig. 8). Of note, even prior to stimulation with hypoxic supernatant (0 d), BM Mø were

**FIGURE 5.** Mø are skewed toward M1 destroyers, rather than M2 healers, in MRL-Faslpr mice after I/R. (A) Renal Mø (F4/80+ and CD68+) in MRL-Faslpr and lupus-resistant (BALB/c and B6) male mice were detected by immunostaining at increasing times after I/R. We initiated I/R at 6 wk of age. (B1–3) Intrarenal M1 and M2 Mø evaluated in MRL-Faslpr and B6 at increasing times after I/R, using flow cytometry and immunostaining. (B1) Diagram analysis using flow cytometry. (B2) Representative FACS plots by gating on the CD45+CD11b+F4/80hi/low cells are shown. (B3) Numbers of F4/80hi and F4/80low cells. (B4) Left, M1-like (TNF-α+/F4/80low/hi, CD80+/F4/80low/hi, CD86+/F4/80low/hi, Ly6C+/-/F4/80low/hi, IL-10+/F4/80low/hi, IL-10+/F4/80low/hi, IL-10+/F4/80low/hi) Mø were evaluated at increasing times after I/R, using flow cytometry. Right, M1-like (iNOS+F4/80+) and M2-like (CD206+F4/80low/hi) Mø were evaluated at increasing times after I/R, using immunostaining. Representative photomicrographs at day 20 after I/R (original magnification ×40). Data represent means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
more skewed toward the M1 phenotype in MRL-Fas lpr mice and more skewed toward the M2 phenotype in lupus-resistant mice (Fig. 8). In fact, prior to stimulation, MRL-Fas lpr BM Mø appear activated (large and elongated) compared with similarly prepared BM Mø from lupus-resistant mice (Fig. 8). As the same mediators released from hypoxic TECs skew Mø toward an M2 phenotype in lupus-resistant mice, but toward an M1 phenotype in MRL-Faslpr mice, this suggests that Mø in MRL-Faslpr mice are inherently poised to destroy, not repair, after transient injury.

**Discussion**

We now report that transient ischemic kidney injury (I/R) elicits defective renal repair and nonresolving inflammation leading to early-onset lupus nephritis in preclinical MRL-Faslpr mice. However, defective renal repair is not unique to MRL-Faslpr mice, as flawed I/R elicited healing is a feature of other lupus-susceptible mice (Sle 123) and MRL mice without the Faslpr mutation (MRL-++). Although CSF-1 hastens healing after I/R in lupus-resistant mice, CSF-1 impairs renal repair, exacerbates nonresolving inflammation, and triggers early-onset lupus nephritis in MRL-Faslpr mice. In probing further, we find that Mø in MRL-Faslpr mice fail to shift from an M1 “destroyer” to M2 “healer” phenotype. Indeed, Mø in MRL-Faslpr mice are inherently skewed toward the M1 destroyer phenotype. The time-related balance of M1 to M2 Mø shifts toward the M2 healer phenotype in lupus-resistant mice after I/R, but M1 continue to dominate in lupus-susceptible (MRL-Faslpr and Sle 123) mice. Moreover, hypoxic TECs release mediators that stimulate the expansion of BM Mø in MRL-Faslpr mice inherently poised to destroy the kidney. The data taken together indicate that ischemic injury induces CSF-1 in injured TECs that foster the expansion of aberrant Mø (M1 phenotype), which mediate defective renal repair and nonresolving inflammation, thereby hastening the onset of lupus nephritis in MRL-Faslpr mice.

**FIGURE 6.** Mø are skewed toward M1 destroyers, rather than M2 healers, in other lupus-susceptible mice that express Fas. Intrarenal Mø phenotype (M1/M2) analysis in Sle 123 female mice 20 d after I/R. We initiated I/R at 10 wk of age. Lupus-resistant (B6) female mice after I/R served as a negative control, and MRL-Faslpr female mice after I/R served as a positive control. Control mice were 6 wk of age at initiation of I/R. M1 (TNF-α/F4/80low/hi, CD80/F4/80low/hi, CD86/F4/80low/hi, Ly6C hi/F4/80low/hi) and M2 (CD206/F4/80low/hi, IL-10/F4/80low/hi, IL4R/F4/80low/hi) Mø were evaluated by flow cytometry after I/R (day 20). Data represent means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 7.** During spontaneous lupus nephritis (MRL-Faslpr), M1 increase more robustly than M2 Mø in MRL-Faslpr kidneys. B6 mice at 20 d after I/R served as control. Prenephritic and nephritic MRL-Faslpr mice were 6 wk and 5 mo of age, respectively. B6 mice were 6 wk of age at initiation of I/R. (A) Intrarenal Mø phenotype M1 (F4/80 low) cells and M2 (F4/80 hi) cells evaluated by flow cytometry. Representative FACS plots gating on the CD45⁺ CD11b⁺ F4/80⁺ are shown. (B) Mø were evaluated using the following markers: M1 (TNF-α/F4/80low/hi, CD80/F4/80low/hi, CD86/F4/80low/hi, Ly6C hi/F4/80low/hi) and M2 (CD206/F4/80low/hi, IL-10/F4/80low/hi, IL4R/F4/80low/hi) (flow cytometry). Data represent means ± SEM and are representative of three separate experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
Growing evidence suggests that transient injury may trigger defective repair and lupus in a variety of tissues, including the kidney. For example, transient, low-level UVB exposure to the skin induces CSF-1 in keratinocytes, thereby recruiting Mø that mediated inflammation leading to repair in lupus-resistant mice, but triggering cutaneous (discoid) lupus in MRL-Fas<sup>lpr</sup> mice (36). As UVB incites similar levels of CSF-1 in keratinocytes, this suggests that the leukocytes in the skin responding to CSF-1 in lupus-resistant mice mediate healing, whereas those in MRL-Fas<sup>lpr</sup> mice mediate destruction. In fact, aberrant Mø responding to CSF-1 after sunlight exposure may explain the mechanism by which sunlight triggers Mø-mediated CSF-1–dependent cutaneous lupus in lupus-susceptible patients. Thus, transient injuries leading to defective repair, nonresolving inflammation, and early-onset lupus in the kidney and skin in MRL-Fas<sup>lpr</sup> mice are apparently analogous. Moreover, our studies indicate that defective repair is not

**FIGURE 8.** Hypoxia-injured TECs release mediators that skew Mø toward M1 in MRL-Fas<sup>lpr</sup> and lupus-resistant (BALB/c) mice, while repairing TECs release mediators that skew Mø toward M2 in lupus-resistant, but not MRL-Fas<sup>lpr</sup>, mice. Scheme depicting experimental design using hypoxic C1 TEC supernatant (effectors) and BM Mø (responders). Supernatant of C1 TECs collected at 1 d and 5 d after hypoxia. BM Mø incubated with C1 supernatant for 24 h. M1 (TNF-α and CD86) and M2 (IL-10 and CD206) phenotypes evaluated by FACS analysis (left panels). M1 (TNF-α and iNOS) and M2 (arginase-1 and IL-10) evaluated by real time PCR. Representative photomicrographs of MRL-Fas<sup>lpr</sup> and BALB/c BM Mø stimulated with supernatant from C1 TECs after hypoxia (original magnification ×40). BM Mø were isolated from male mice at 6 wk of age. Data represent means ± SEM.
unique to MRL-Faslpr mice but is a feature of other lupus-susceptible mice, including the Sle123 and MRL-+++. This observation is consistent with tape stripping, another form of transient injury, leading to repair in lupus-resistant mice and, conversely, inciting chronic skin lesions with features of cutaneous lupus in lupus-susceptible NZB × NZW F1 mice (37). It appears that transient injury in lupus-susceptible mice leads to nonresolving inflammation and early-onset lupus.

The MRL background, and not the absence of Fas, is linked to defective repair after I/R. We show that renal repair is defective in preclinical MRL-++ mice (Fas intact), and this defect is more pronounced in preclinical MRL-++ mice that are aged closer to the onset of clinical lupus nephritis. As defective repair is less pronounced in age-matched (6 wk), preclinical MRL-++ compared with MRL-Faslpr mice, our experiments differ from those indicating that blocking Fas ligand in lupus-resistant mice (B6) attenuates injury (38). However, as the MRL background is central to defective renal repair after I/R, it is possible that the impact of signaling through Fas is masked or altered in the context of attenuating injury (38). Nevertheless, our data, along with other reports (42, 43), challenge this concept. Thus, defective renal repair leading to early-onset lupus nephritis is linked to the MRL background.

Ample evidence suggests that Mø in lupus-susceptible mice are aberrant. Mø in MRL-Faslpr mice hyperproliferate to CSF-1 (5); display enhanced MHC Ia expression (6); have a broad-range phagocytic defect, including diminished clearance of RBCs, yeast, and apoptotic cells, epithelial cells (G. Tesch and V. R. Kelley, unpublished observations), and T cells (25); aberrantly express cytokines (44–46); and exhibit abnormal Rho-dependent cytoskeletal organization (47). We now report that these aberrant Mø fail to repair renal injury. We detected a shift in the dominance of M1 Mø at the initiation of renal injury to M2 Mø during repair after I/R in lupus-resistant mice. This finding is consistent with the switch from a proinflammatory (M1) to a reparative (M2) phenotype during the transition from tubule injury to tubule repair (20). We now report that nonresolving inflammation and early-onset lupus are mediated by the failure of a predominance of M1 Mø to shift toward M2 Mø in the kidney. As Mø from MRL-Faslpr mice proliferate more robustly to CSF-1 than do those from lupus-resistant mice, CSF-1 generated by injured tubules expands the M1 rather than M2 Mø, and thereby fosters destructive inflammation leading to early-onset lupus nephritis. This defective shift from M1 to M2 may be a general feature of intrarenal Mø in lupus-susceptible hosts following injury, as intrarenal Mø in Sle123 mice respond similarly after renal injury. Moreover, abnormal mononuclear phagocytes have been identified in other models of lupus nephritis. In particular, mononuclear phagocytes with an aberrant activation profile contribute to tissue damage by mediating local intrarenal inflammation in lupus-susceptible NZB/W mice (48). We appreciate that the role of M1 and M2 Mø highlighted in our study may be only one facet of a more widespread disturbance of innate immunity in lupus-susceptible mice and individuals. This hypothesis is currently being studied.

Although intrarenal Mø are distinct in lupus nephritis, their phenotypes are not consistent in all models. We report a skewing toward M1 (F4/80low), rather than M2 (F4/80hi), phenotypes in MRL-Faslpr and Sle123 mice during lupus nephritis incited by I/R and in spontaneous lupus nephritis in MRL-Faslpr mice. In contrast, intrarenal Mø in an induced model of lupus nephritis (activation lymphocyte-derived DNA) polarize Mø toward an M2b phenotype (49). As M2b Mø are induced by exposure to immune complexes or TLR (19), this may suggest that the pathogenesis of lupus nephritis in MRL-Faslpr and Sle123 mice versus activation lymphocyte-derived DNA–induced lupus nephritis differ. Moreover, Mø in the kidney during lupus nephritis in NZB/W mice acquire a high expression of F4/80 (48). As lupus nephritis may be triggered by a variety of mechanisms, it is plausible that dissimilar Mø phenotypes reflect differences within patient populations with distinct mechanisms. Moreover, recognizing that the methods used for phenotyping Mø are not precisely standardized, we are not able to make exacting comparisons with studies from other laboratories. Indeed, a standardized, detailed comparison of intrarenal Mø phenotypes in multiple mouse models of lupus nephritis and human lupus nephritis will likely uncover a stratification of the pathogenesis leading to novel therapeutic strategies for this illness.

In sum, we speculate that nonresolving inflammation triggering lupus nephritis results from aberrant Mø poised to destroy—rather than repair—infected kidneys. To more fully understand the mechanism responsible for escalating destructive, rather than reparative, Mø after renal injury, we are exploring whether defective switching is a result of inherently abnormal Mø polarization in lupus-susceptible mice, or one facet of a broader defect in innate immunity. However, we wish to point out that it is also possible that destructive Mø persist because other triggers continually provoke nonresolving inflammation. We anticipate that future studies directed toward clarifying this “chicken and egg” problem will elucidate the pathogenesis and thereby provide novel therapeutic approaches to human lupus nephritis.

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Disclosures

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