Monocyte Chemoattractant Protein 1–dependent Leukocytic Infiltrates Are Responsible for Autoimmune Disease in MRL-Faslpr Mice

By Gregory H. Tesch,* Stefanie Maifert,* Andreas Schwarting,* Barrett J. Rollins,‡ and Vicki Rubin Kelley*

From the *Laboratory of Molecular Autoimmune Disease, Renal Division, Brigham and Women's Hospital, Boston, Massachusetts 02115; and the ‡Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115

Summary

Infiltrating leukocytes may be responsible for autoimmune disease. We hypothesized that the chemokine monocyte chemoattractant protein (MCP)-1 recruits macrophages and T cells into tissues that, in turn, are required for autoimmune disease. Using the MRL-Faslpr strain with spontaneous, fatal autoimmune disease, we constructed MCP-1–deficient MRL-Faslpr mice. In MCP-1–intact MRL-Faslpr mice, macrophages and T cells accumulate at sites (kidney tubules, glomeruli, pulmonary bronchioli, lymph nodes) in proportion to MCP-1 expression. Deleting MCP-1 dramatically reduces macrophage and T cell recruitment but not proliferation, protects from kidney, lung, skin, and lymph node pathology, reduces proteinuria, and prolongs survival. Notably, serum immunoglobulin (Ig) isotypes and kidney Ig/C3 deposits are not diminished in MCP-1–deficient MRL-Faslpr mice, highlighting the requirement for MCP-1–dependent leukocyte recruitment to initiate autoimmune disease. However, MCP-1–deficient mice are not completely protected from leukocytic invasion. T cells surrounding vessels with meager MCP-1 expression remain. In addition, downstream effector cytokines/chemokines are decreased in MCP-1–deficient mice, perhaps reflecting a reduction of cytokine-expressing leukocytes. Thus, MCP-1 promotes MRL-Faslpr autoimmune disease through macrophage and T cell recruitment, amplified by increasing local cytokines/chemokines. We suggest that MCP-1 is a principal therapeutic target with which to combat autoimmune diseases.

Key words: mouse • kidney • lung • chemokine • gene disruption

Infiltrating mononuclear leukocytes may be responsible for autoimmune tissue injury (1, 2). The migration of leukocytes through vessels and beyond the vascular compartment is dependent in part on small chemoattractant proteins called chemokines. Within the chemokine family, a series of reports suggest that monocyte chemoattractant protein (MCP)-1 may be responsible for inflammation in tissues during autoimmune disease. This is based on (a) MCP-1 tissue expression in human systemic lupus erythematosus, rheumatoid arthritis (3–5), and experimental mouse models of lupus (NZB/W mice) and allergic encephalomyelitis (6–7); (b) MCP-1 chemoattraction for monocytes and memory T cells (8–10); and (c) MCP-1 regulation of leukocyte function via modulation of adhesion molecule expression and T cell activation and proliferation (11–14). Thus, MCP-1 may be the principal chemokine responsible for initiating autoimmune tissue damage.

Strategies that neutralize or eliminate MCP-1 are protective in induced models of inflammation (15–19). MCP-1 antibody neutralization markedly reduces infiltrating macrophages and T cells in induced models of kidney and lung injury (16–19). However, antibody-based therapies have limitations; they do not necessarily deplete the target molecule and may even initiate harmful immune reactions. Perhaps the most convincing evidence supporting the requirement for MCP-1 in inflammation is illustrated in MCP-1–deficient mice (20). For example, MCP-1–deficient mice mount meager responses to inflammatory or immune stimuli, as evidenced in thioglycolate-elicited peritonitis, delayed-type hypersensitivity responses, and pulmonary granulomas (20). Furthermore, using a form of rapidly progressive kidney disease responsible for tubular and glomerular injury, nephrotoxic serum nephritis (N SN), we established that MCP-1–deficient B6/129 mice are spared from tubular but not glomerular destruction (21). This structure-spe-
Lymphadenopathy, splenomegaly, and autoimmune injury are caused by the infiltration of mononuclear leukocytes that is responsible for tissue destruction in murine lupus (MRL-Fas<sup>lpr</sup>) studies. The phenotypic expression of autoimmune disease is sufficiently long to tease apart pathogenesis but steady progressive, fatal, and shares features with human systemic lupus erythematosus (23). Furthermore, the tempo of disease is sufficiently long to tease apart pathogenesis but short enough to be economically feasible for experimental studies. The phenotypic expression of autoimmune disease in MRL-Fas<sup>lpr</sup> mice is characterized by a large tissue infiltration of mononuclear leukocytes that is responsible for lymphadenopathy, splenomegaly, and autoimmune injury in multiple tissues, including the kidney, lung, and skin (23). Kidney disease in MRL-Fas<sup>lpr</sup> mice is fatal and complex and consists of glomerular, tubular/interstitial, and vascular components mediated by infiltrating macrophages and T cells. Furthermore, the pathogenic events in each component within the kidney are distinctive. We reason that chemokines that recruit mononuclear leukocytes into the kidney and other tissues targeted for autoimmune disease are responsible for eliciting this cascade of events culminating in fatal autoimmune disease. Therefore, we hypothesize that MCP-1 is responsible for recruiting macrophages and T cells and thereby initiating disease in the kidney and other tissues undergoing autoimmune disease in MRL-Fas<sup>lpr</sup> mice. To test this hypothesis, we have constructed an MCP-1-deficient MRL-Fas<sup>lpr</sup> strain, evaluated pathology (kidney, lung, skin, lymph nodes, and spleen) and survival, and determined the mechanism responsible for leukocytic accumulation within these tissues.

**Materials and Methods**

Mice. MRL/Mp j<sup>lpr</sup> (<MRL<sup>lpr</sup>), MRL/Mp j-Fas<sup>lpr</sup>Fas<sup>+</sup> (MRL-Fas<sup>lpr</sup>), C3H/FeJ, and C57BL/6 mice were purchased from The Jackson Laboratory. MRL-1/1-intact and -deficient B6/129 mice (129SvJ × C57BL/6F<sub>1</sub>) were constructed as described (20). Control MRL-1/1-intact B6/129 mice were derived from matings of mice heterozygous for the disrupted allele. All mice were maintained in a pathogen-free animal facility.

Generating MCP-1-deficient MRL-Fas<sup>lpr</sup> Mice. MCP-1-deficient (MCP-1<sup>−/−</sup>) MRL-Fas<sup>lpr</sup> mice were created by a series of genetic backcrosses using the cross-backcross-intercross scheme. MRL-Fas<sup>lpr</sup> mice were mated with MRL-1/1-intact (129SvJ × C57BL/6) mice to yield heterozygous F1 offspring. We intercrossed F1 mice and screened the progeny by PCR amplification of tail genomic DNA for the Fas<sup>lpr</sup> mutation and MCP-1 using specific primers (21, 24). Double homozygotes (Fas<sup>lpr</sup>Fas<sup>lpr</sup>MCP-1<sup>−/−</sup>) N1F1 progeny were backcrossed to MRL-Fas<sup>lpr</sup> mice. B1 progeny, homozygous for the Fas<sup>lpr</sup> mutation and heterozygous for MCP-1 (MCP-1<sup>1+/−</sup>), were intercrossed, and mice homozygous for the disrupted MCP-1 gene were selected by PCR typing for continued backcrossing. After three generations of backcross-intercross matings, this breeding scheme generated a colony of MRL-Fas<sup>lpr</sup> mice (94% MRL-Fas<sup>lpr</sup> background) homozygous and heterozygous for the disrupted MCP-1 gene. We analyzed the third generation, as we have previously established that there are sufficient MRL-Fas<sup>lpr</sup> background genes to result in phenotypic changes characteristic of the wild-type MRL-Fas<sup>lpr</sup> strain (25). In addition, we compared sex-matched littersmates to minimize variability. The MRL-1/1-intact MRL-Fas<sup>lpr</sup> mice were termed MCP-1-deficient, whereas the MCP-1<sup>1+/−</sup>MCP-1<sup>−/−</sup> MRL-Fas<sup>lpr</sup> mice are termed MCP-1-intact MRL-Fas<sup>lpr</sup> mice.

Proteinuria. Urine protein levels in MRL-1/1-intact and -deficient MRL-Fas<sup>lpr</sup> mice were assessed semiquantitatively by dipstick analysis (Albustix; Bayer Diagnostic Division) on a monthly basis beginning at 2 mo of age. On the day of analysis, dipstick proteinuria measurements were taken from individual mice in the morning and evening. If the measurements were inconsistent, the animal was reassessed the following day.

Lymphadenopathy, Splenomegaly, and Skin Lesions. Protruding lymph nodes (cervical, brachial, and inguinal) and skin lesions were assessed monthly beginning at 3 mo of age. Lymph node score based on palpable nodes: 0 = none; 1 = small, at one site; 2 = moderate, at two different sites; and 3 = large, at three or more different sites. Skin lesion score by gross pathology: 0 = none; 1 = small (face or ears); 2 = moderate, <2 cm (face, ears, and back); and 3 = severe, >2 cm (face, ears, and back). Splenomegaly was determined by spleen weights.

Renal Pathology. Kidneys were fixed in 10% formalin for 24 h at 4°C. Paraffin sections (4 μm) were stained with hematoxylin and periodic acid Schiff’s (PAS) reagent. We evaluated glomerular pathology by assessing 50 glomerular cross sections (gcs) per kidney and scored each glomerulus on a semiquantitative scale: 0 = normal (35–40 cells/gcs); 1 = mild (glomeruli with few lesions showing slight proliferative changes, mild hypercellularity [41–50 cells/gcs], and/or minor exudation); 2 = moderate (glomeruli with moderate hypercellularity [50–60 cells/gcs], including segmental and/or diffuse proliferative changes, hyalinosis, and moderate exudate); and 3 = severe (glomeruli with segmental or global sclerosis and/or exhibiting severe hypercellularity [>60 cells/gcs], necrosis, crescent formation, and heavy exudation). Damaged tubules (percent consisting of dilatation and/or atrophy and/or necrosis) were determined in 400 randomly selected renal cortical tubules per kidney (<400). Perivascular cell accumulation was determined semiquantitatively by scoring the number of cell layers surrounding the majority of vessel walls (score: 0 = none; 1 = <5; 2 = 5–10; and 3 = >10). Scoring was evaluated using coded slides.

Lung Pathology. The lungs were fixed and inflated with 10% formalin, and paraffin sections (4 μm) were stained with hematoxylin and PAS reagent. Perivascular and peribronchial infiltrates were assessed semiquantitatively in >20 vessels per section and in >20 bronchioli per section (number of cell layers surrounding
the majority of vessels or bronchioli: 0 = none; 1 = 1–3; 2 = 3–6; and 3 = >6).

A nitrite. The following primary antibodies were used for immunostaining: rat anti–mouse CD4 IgG2a clone R M4-5 (Pharmingen) to detect CD4 T cells; rat anti–mouse CD8a (Ly-2) IgG2a clone 53-6.7 (Pharmingen) to detect CD8 T cells; rat anti–mouse CD45R/B220 IgG2a clone RA3-682 (Pharmingen) to detect CD4/CD8 T cells; rat anti–mouse CD21/35 IgG2b clone 7G6 (Pharmingen) to detect B cells; rat anti–mouse monocyte/macrophage IgG2b clone M O M A-2 (BioSource International) and rat anti–mouse macrophage IgG2b (prepared from F4/80 hybridoma supernatant; American Type Culture Collection number HB 198) to detect macrophages; rabbit anti–mouse M CP-1 IgG (Serotec Ltd.) to detect MCP-1; mouse anticytokeratin peptide 18 IgG1 clone CY-90 (Sigma Chemical Co.) to detect epithelial cells; fluorescein-conjugated mouse anti–PCNA (proliferating cell nuclear antigen) IgG1 clone 19F4 (Boehringer Mannheim) to detect proliferating cells; fluorescein-conjugated goat anti–mouse IgG (Organon Teknika) to detect mouse IgG; and fluorescein-conjugated goat anti–mouse C3 (Organon Teknika) to detect mouse complement C3. The negative isotype control antibodies for immunostaining were rat IgG2a clone R 35-95, rat IgG2b clone R 35-38, mouse IgG1 clone M OC-P-21 (Pharmingen), and normal rabbit IgG (Sigma Chemical Co.). The secondary antibodies for immunostaining were biotin-conjugated rabbit anti–rat IgG and biotin-conjugated goat anti–rabbit IgG (Vector Labs.). ELISA analysis of serum Ig (total Ig, IgG, IgG1, IgG2a, IgG2b, and IgG3) was performed using isotype-specific, goat anti–mouse capture antibodies, and alkaline phosphatase–conjugated goat anti–mouse IgG2b antibodies to detect mouse antibodies (Southern Biotechnology Associates Inc.).

Identifying Infiltrating Cells. To analyze kidney- and lung-infiltrating T cells and interstitial macrophages, cryostat tissue sections (4 μm) were fixed in ethanol at 4°C for 10 min and immunostained with CD4, CD8, B220, and F4/80 antibody as previously described (26) using the avidin–biotin–peroxidase detection system (Vector Labs.). Glomerular macrophages were identified in 4 μm acetone-fixed tissue sections using MOMA-2 antibody as previously reported (16). Cells infiltrating within and around (peri) glomeruli were assessed by counting the number of labeled cells in 20 randomly selected glomeruli per section. The cells infiltrating around tubules were enumerated in 400 randomly selected tubules per section. Macrophages and T cells within glomeruli, adjacent to glomeruli, or adjacent to cortical tubules were expressed as a cell index (mean cell number/glomeruli × glomeruli/section; mean cell number/cortical tubule × cortical tubules/section). Peribronchial and perivascular macrophages and T cells (CD4, CD8, B220) in the lungs were assessed by measuring the unit area stained/unit length of bronchiole/vessel (five per animal) with a micrometer.

Detecting MCP-1 in Tissues. To detect MCP-1, formalin-fixed sections were deparaffinized and incubated with 20% normal goat serum for 30 min. Tissue sections were incubated with M CP-1 antibody (10 μg/ml) in 1% BSA overnight at 4°C. Bound primary antibody was labeled with biotin-conjugated goat anti–rabbit IgG for 1 h and subsequently detected using the avidin–biotin–peroxidase system (Vector Labs.). We confirmed MCP-1 antibody specificity using two methods: (a) M CP-1 was not detectable in tissue sections from M CP-1–deficient mice (negative control), and (b) M CP-1 was expressed in Western blots of cell lysates from LPS-stimulated bone marrow macrophages and mesangial cells in M CP-1–intact but not M CP-1–deficient mice, as previously reported (21). Glomerular MCP-1 immunostaining was assessed by the same scoring system used to detect glomerular infiltrating cells. We analyzed M CP-1 in cortical tubules in 400 tubules per cross section. We enumerated the kidney and pulmonary vessels and bronchioli that expressed M CP-1 in each section and recorded values as the percent positive.

Serum Immunoglobulins Profile. ELISA plates were coated overnight at 4°C with 5 μg/ml goat anti–mouse IgG capture antibodies (against total Ig, IgM, IgG1, IgG2a, IgG2b, and IgG3) in 0.1 M carbonate buffer, pH 9.4. Wells were blocked for 1 h with assay diluent (2% BSA in 0.1 M borate buffer, pH 8.0). We then added Ig standards to the plates (50 μl/well), starting at 1 μg/ml and performing a series of threefold dilutions, and assessed serum samples using serial (threefold) dilutions starting at 1:100 or 1:1,000. Standards and serum samples were incubated overnight at 4°C, and bound Ig was detected with goat anti–mouse detection antibodies conjugated with alkaline phosphatase and enzymatically developed by incubating with Sigma 104 phosphatase substrate in 9.6% diethanolamine and 0.1 M MgCl2, pH 9.8 (Sigma Chemical Co.). A absorbance was measured at 405 nm. IgG and C3 in Kidneys. To examine IgG and C3 deposits in the kidney, we incubated cryostat-sectioned tissues (4 μm) with 20% normal goat or rabbit serum (30 min), followed by fluorescein-conjugated antibodies detecting mouse IgG or mouse C3 (30 min), washing, and mounting with Vectashield (Vector Labs.). We assessed immunofluorescence staining by titrating the antibodies on serial tissue sections using twofold dilution steps (1:100–1:25,600).

A apoptotic and Proliferating Cells in Situ. Apoptotic cells were identified in tissue sections (4 μm) using the terminal deoxynucleotidyl transferase–mediated dUTP–biotin nick-end labeling (TUNEL) method and immunoperoxidase staining (In Situ Cell Death Detection Kit; Boehringer Mannheim). Proliferating cells were detected by PCNA immunostaining as previously described (27). Sections were counterstained with PAS reagent to determine morphology. Apoptosis in glomeruli and tubules was assessed by counting the number of T U N E L–labeled cells in 50 glomeruli per section and 500 cortical tubules per section, respectively.

PCR Detection of MCPs and Cytokines in the Kidney. Total RNA was extracted from the snap-frozen renal cortex of half a kidney using R NA zol B (Tel-Test Inc.) and reverse transcribed using oligo–dT and the SuperScript II DNA preamplification kit (GIBCO BRL). The resulting reverse transcription product was the cDNA template for PCR analysis. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression was detected as a 500-bp product resulting from PCR with specific oligonucleotide primers (antisense, 5'-CAAAGGTTGCTCAGGATGACC-3'; and sense, 5'-GGTTGAGGTCGAGTCAACG-3', reference 21). The chemokines M CP-1, M CP-3, and M CP-5 were detected as 350-, 370-, and 390-bp PCR products, respectively, using specific oligonucleotide primers (M CP-1 antisense, 5'-GCTTGGAGTGGTGTGGAAGA-3'; M CP-1 sense, 5'-CTCACCTGCTGCTAC-3'; M CP-3 antisense, 5'-CACATTCTCTACAGACAGC-3'; and sense, 5'-AGCTACAGAGATGAGCAGC-3'; M CP-5 antisense, 5'-CTCCTTTATCCAGTATGGCC-3'; and sense, 5'-TTTCCTTTCACCACATGGAG-3', references 28–30). The cytokines IFN-γ and CSF-1 were detected as 500- and 245-bp PCR products using specific primers (IFN-γ sense, 5'-CACGGGCACACGTGTTAAGCC-3'; IFN-γ antisense, 5'-CTTATGCGAATTCTTCCC-3'; CSF-1 sense, 5'-CACATGGATTGGAATGACA-3'; and CSF-1 antisense, 5'-CAGCTGGTTCAGTTAATGGCA-3'; references 31 and 32). The PCR primers and conditions were chosen so that the MCP and GAPDH products were amplified with equal efficiency.

Statistics. Data was analyzed using the Kruskal–Wallis test for

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Results

Increased Expression of MCP-1 in MRL-Fas<sup>pr</sup> Tissues during Autoimmune Disease. MCP-1 is upregulated in MRL-Fas<sup>pr</sup> kidneys, lungs, and lymph nodes during autoimmune disease. First, we detected an increase (threefold) in MCP-1 mRNA within the renal cortex of wild-type pure MRL-Fas<sup>pr</sup> mice as early as 2 mo of age, as compared with the C57BL/6 strain with normal kidneys. MCP-1 mRNA increased further within the renal cortex (six- to eightfold) with advancing renal injury in these MRL-Fas<sup>pr</sup> mice (Fig. 1). We localized MCP-1 expression to several structures within the MRL-Fas<sup>pr</sup> kidney and determined that the rank order of tissue expression was tubules → glomeruli → vasculature. MCP-1 in MRL-Fas<sup>pr</sup> kidneys (5–6 mo of age) was almost exclusively localized within the TECs. More specifically, MCP-1 was within the proximal TECs (brush border histologic identification), whereas the distal TECs (histologic identification) were only weakly positive or negative. MCP-1 expression in MRL-Fas<sup>pr</sup> glomeruli was determined to be predominantly in epithelial podocytes, based on the morphological positioning of MCP-1-stained cells in glomeruli after histologic counterstaining (Fig. 2 b and Fig. 3 a), whereas lesser amounts localized within the epithelial cells and macrophages within crescents (two to three cells per crescent). Finally, MCP-1 in the vasculature within endothelial and smooth muscle cells was minimal (Fig. 3 a, insert). It is noteworthy that few (1–2%) infiltrating cells in the renal interstitium express MCP-1. Thus, kidney MCP-1 expression is almost exclusively within parenchymal cells and in epithelial cells in particular, rather than infiltrating cells.

MCP-1 is expressed in other tissues in MRL-Fas<sup>pr</sup> mice during autoimmune disease. Similar to MCP-1 in the kidney, MCP-1 in the lungs is predominantly (>90%) expressed in epithelial cells (bronchioli; Fig. 3 b) and is weakly detected within vascular endothelial and interstitial cells in MRL-Fas<sup>pr</sup> mice (5 mo of age). Massively enlarged lymph nodes caused by an influx of T cells are characteristic of MRL-Fas<sup>pr</sup> autoimmune disease (23). MCP-1 is readily detected in cells surrounding lymphatic vessels within these enlarged lymph nodes (inguinal, cervical) in MRL-Fas<sup>pr</sup> mice (5 mo of age; Fig. 3 c). As anticipated, MCP-1 was not detected in MCP-1-deficient MRL-Fas<sup>pr</sup> tissues (Fig. 3, d–f). Taken together, these data indicate that MCP-1 is mainly expressed by parenchymal cells in multiple tissues targeted for autoimmune injury in MRL-Fas<sup>pr</sup> mice.

MCP-1-deficient MRL-Fas<sup>pr</sup> Mice Survive Longer than MCP-1-intact MRL-Fas<sup>pr</sup> Mice and Are Protected from Proteinuria. MCP-1-deficient MRL-Fas<sup>pr</sup> mice have a prolonged life span as compared with MCP-1-intact MRL-Fas<sup>pr</sup> strains. The vast majority of MCP-1-deficient MRL-Fas<sup>pr</sup> mice (75%) remained alive at 300 d, as compared with a surviving minority of MCP-1-intact (MCP-1<sup>1/2</sup>, 17% and MCP-1<sup>1/4</sup>, 44%) MRL-Fas<sup>pr</sup> mice (Fig. 4 a; P < 0.0001). Notably, the mortality (50%) of the MCP-1<sup>1/4</sup> MRL-Fas<sup>pr</sup> strain third generation was 7 mo of age, which is similar to that of the pure wild-type MRL-Fas<sup>pr</sup> strain (6 mo of age).
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and dissimilar to that of C57BL/6J and Sv/129 strains (>20 and 16 mo of age, respectively; reference 33).

It is important to note that the spot analysis for protein in fresh individual urine specimens has several limitations, including the sample size (volume) and semiquantitative measurement. However, our confidence in making inferences from this method is enhanced by the large number of mice in each group and sequential monthly analysis. With these caveats in mind, we now report that MCP-1–deficient MRL–Fas<sup>pr</sup> mice are protected from proteinuria (2–6 mo) in comparison to the MCP-1–intact MRL–Fas<sup>pr</sup> strain. The number of surviving MCP-1–intact MRL–Fas<sup>pr</sup> mice declines rapidly after 6 mo of age; therefore, proteinuria at these ages is limited to a subset of MCP-1–intact MRL–Fas<sup>pr</sup> mice, which are more resistant to disease (normal B6/129 wild type). (c) Lymphadenopathy is reduced in MCP-1–deficient compared with –intact strains. (d) Inflammatory skin lesions are reduced in MCP-1–deficient compared with –intact strains. Data mean ± SEM; *P < 0.05; **P < 0.005; and ***P < 0.0001 compared with MCP-1<sup>−/−</sup>.
caveats in mind, we now report that MCP-1-deficient versus MCP-1-intact MRL-Fas<sup>pr</sup> mice are protected from pathological proteinuria. From 2 to 8 mo of age, both the rate of increase and incidence of pathological proteinuria were diminished in MCP-1-deficient MRL-Fas<sup>pr</sup> mice (Fig. 4 b). For example, the vast majority (82%) of MCP-1-deficient MRL-Fas<sup>pr</sup> mice had normal, nonpathologic proteinuria (1<sup>1</sup>1), whereas the majority (62%) of MCP-1-intact MRL-Fas<sup>pr</sup> mice were pathologically proteinuric (2–4<sup>1</sup>1) at 8 mo of age. It should be noted that urinary protein in normal B6/129 wild-type mice is barely detectable (0–1<sup>1</sup>1; data not shown). Thus, MCP-1-deficient MRL-Fas<sup>pr</sup> mice are protected from proteinuria.

Lymphadenopathy and Skin Lesions Are Reduced in MCP-1-deficient MRL-Fas<sup>pr</sup> Mice. We examined whether MCP-1 promotes autoimmune disease in the lymph nodes, spleen, and skin of MRL-Fas<sup>pr</sup> mice from 3 to 8 mo of age. The incidence and severity of lymphadenopathy and skin lesions was reduced but not totally eliminated in MCP-1-deficient as compared with MCP-1-intact MRL-Fas<sup>pr</sup> mice (Fig. 4, c and d). For example, although nearly every MCP-1-intact MRL-Fas<sup>pr</sup> mouse had palpable lymph nodes (94%), most of the MCP-1-deficient mouse lymph nodes were not palpable at 5 mo of age (69%). Similarly, the majority (84%) of MCP-1-deficient mice were spared skin lesions, whereas most (67%) of the MCP-1-intact MRL-Fas<sup>pr</sup> mice had gross skin pathology at 8 mo of age. On the other hand, we did not detect a difference in splenomegaly in MCP-1-deficient (234 ± 64 mg) versus MCP-1-intact MRL-Fas<sup>pr</sup> mice (242 ± 143 and 289 ± 174 mg MCP-1<sup>1/+</sup> and MCP-1<sup>1/-</sup>, respectively; P = 0.2; n = 6 per group) at 5 mo of age.

Reduced Kidney and Lung Pathology in MCP-1-deficient MRL-Fas<sup>pr</sup> Mice. To determine if MCP-1 is required for kidney and lung pathology, we compared MCP-1-deficient and –intact MRL-Fas<sup>pr</sup> mice killed at 5 mo of age. Renal (tubular, glomerular) and pulmonary pathology was reduced in MCP-1-deficient as compared with MCP-1-intact MRL-Fas<sup>pr</sup> mice (Figs. 5 and 6). The reduction in tubular and glomerular pathology assessed histologically was dramatic (50–70%) in MCP-1-deficient MRL-Fas<sup>pr</sup> kidneys (Fig. 6, a and b; P < 0.05). In particular, MCP-1-deficient MRL-Fas<sup>pr</sup> mice had markedly diminished peritubular infiltrate, tubular atrophy, glomerular hypercellularity, glomerulosclerosis, and crescent formation. We further evaluated the level of tubular and glomerular damage by identifying apoptotic cells. The number of apoptotic cells was reduced in MCP-1-deficient versus MCP-1-intact MRL-Fas<sup>pr</sup> kidneys. The majority of apoptotic TECs diminished from 4.2 ± 0.3% per section in MCP-1-intact to 2.0 ± 0.6% per section in MCP-1-deficient MRL-Fas<sup>pr</sup> kidneys (P < 0.05; n = 6 per group). Similarly, the number of apoptotic glomerular cells was reduced in MCP-1-deficient versus MCP-1-
in MCP-1–deficient MRL-Faslpr mice there was a reduction in tubular and glomerular pathology compared with the MCP-1–intact MRL-Faslpr kidneys (P < 0.05; n = 6; data not shown). In addition, fewer (5/11) MCP-1–deficient MRL-Faslpr mice, as compared with MCP-1–intact MRL-Faslpr mice (12/16), died with severe proteinuria (3–4+). Thus, even in the MCP-1–deficient and MCP-1–intact MRL-Faslpr mice that eventually die, the extent of renal injury is less severe in the MCP-1–deficient MRL-Faslpr strain.

Decreased Infiltrating Cells in the Kidney (Macrophages and T Cells) and Lungs (Macrophages) in MCP-1–deficient versus MCP-1–intact MRL-Faslpr Mice. There was a reduction in the number of macrophages and T cells (CD4 and CD8 but not B220) in MCP-1–deficient versus MCP-1–intact MRL-Faslpr kidneys. The majority of macrophages and T cells localized in the interstitium adjacent to tubules and glomeruli, whereas fewer were identified within glomeruli. We noted the largest (50–72%) decline in macrophages and T cells in the MCP-1–deficient MRL-Faslpr strain surrounding tubules (P < 0.01; Fig. 7 a). Although intraglomerular macrophages were reduced (34%) in MCP-1–deficient MRL-Faslpr kidneys (P < 0.005; Fig. 7 a), the numbers of intraglomerular T cells (CD4, CD8, B220) were not diminished (P = 0.2). The reduction in glomerular macrophages correlated with diminished glomerular morphologic damage (intraglomerular, r = 0.78; periglomerular, r = 0.77; P < 0.005). The absence of MCP-1 in MRL-Faslpr kidneys reduced the number of infiltrating macrophages surrounding bronchioi but not surrounding vessels (Fig. 7 d). In contrast to the reduction in the number of kidney-infiltrating T cells in MCP-1–deficient versus -intact MRL-Faslpr mice, the number of T cells (CD4, CD8, B220) in the lungs was not reduced (P = 0.2; Fig. 7 d).

A Reduction in Macrophages and T Cells in MCP-1–deficient Kidneys and Lungs Did Not Result from Decreased Local Proliferation. We determined if the decreased accumulation of macrophages and T cells in MCP-1–deficient MRL-Faslpr kidneys and lungs at 5 mo of age was a result of a decline in local proliferation using in situ detection of PCNA. First, few of the cells surrounding tubules (<1%), glomeruli (<1%), and bronchioi (<10%) were proliferating in the MCP-1–intact MRL-Faslpr strain. The number of PCNA+ cells was not reduced in the MCP-1–deficient MRL-Faslpr strain (P = 0.2; n = 6). Second, there were substantially more proliferating cells surrounding vessels (>20%) com-
pared with proliferating cells surrounding glomeruli, tubules, and bronchioles in MCP-1–intact MRL-Fas<sup>−/−</sup> kidneys and lungs (*P* < 0.002, and this number did not decrease in the MCP-1–deficient MRL-Fas<sup>−/−</sup> strain (*P* = 0.1; *n* = 6 per group). Of course, it must be appreciated that the proliferation measurements in tissue sections are a reflection of the level of cell division at the time these tissues were removed. Thus, the reduction in kidney- and lung-infiltrating cells in MCP-1–deficient MRL-Fas<sup>−/−</sup> mice does not appear to result from decreased local proliferation.

**CCR2 Ligand Expression in MCP-1–deficient MRL-Fas<sup>−/−</sup> Kidneys Is Decreased.** MCPs 1–5 are ligands for the MCP-1 receptor (CCR2; reference 22). To examine whether CCR2 ligands other than MCP-1 are upregulated after renal injury, we examined MCP-3 and MCP-5 in MRL-Fas<sup>−/−</sup> kidneys. Renal cortical transcripts of MCP-3 and MCP-5 in MRL-Fas<sup>−/−</sup> as compared with C57BL/6 mice were increased (twofold) in advance of overt renal pathology (2 mo of age) and rose further (fourfold) as renal pathology advanced (6 mo of age; Fig. 1). We probed for MCP expression in MCP-1–deficient MRL-Fas<sup>−/−</sup> kidneys. As anticipated, MCP-1 transcripts were not detected in the MCP-1–deficient MRL-Fas<sup>−/−</sup> renal cortex. On the other hand, MCP-3 and MCP-5 transcripts were detected in MCP-1–deficient MRL-Fas<sup>−/−</sup> mice (Fig. 8). However, amounts of MCP-3 and MCP-5 transcripts were 50% lower in MCP-1–intact MRL-Fas<sup>−/−</sup> kidneys (*P* < 0.005; Fig. 8). Thus, MRL-Fas<sup>−/−</sup> mice lacking MCP-1 have reduced intrarenal productions of ligands (MCP-3, MCP-5) that bind to CCR2.

Reduced CSF-1 and IFN-γ T transcripts in MCP-1–deficient MRL-Fas<sup>−/−</sup> Kidneys. We previously established that CSF-1 and IFN-γ transcripts that are upregulated with advancing renal injury in MRL-Fas<sup>−/−</sup> mice are required for autoimmune kidney disease (25, 34, 35). In MCP-1–deficient MRL-Fas<sup>−/−</sup> kidneys, CSF-1 and IFN-γ transcripts were reduced as compared with the MCP-1 intact MRL-Fas<sup>−/−</sup> strain (Fig. 7).
In contrast, CSF-1 and IFN-γ transcripts in MCP-1-deficient and –intact B6/129 mice with normal kidneys were barely detectable (Fig. 8).

Serum- and kidney-deposited IgA are not reduced in MCP-1-deficient MRL-Faslpr mice. To determine if MCP-1 alters the antibody isotype profile in MRL-Faslpr autoimmune disease, we evaluated serum levels of IgGs (total Ig, IgM, IgG1, IgG2a, IgG2b, IgG3) in MCP-1-deficient and –intact MRL-Faslpr mice. We did not detect differences in serum IgGs (amount and isotype) in MCP-1-deficient and –intact MRL-Faslpr strains (Fig. 9a). In addition, we did not detect an alteration in the amount and distribution of IgS within MRL-Faslpr-deficient and –intact MRL-Faslpr kidneys (Fig. 9b). Similarly, complement (C3) deposition was not reduced in MCP-1-deficient as compared with MCP-1-intact MRL-Faslpr glomeruli (data not shown). Thus, MCP-1 does not regulate circulating or kidney-depositing IgGs in MRL-Faslpr mice.

Discussion

In this report, we tested the hypothesis that a specific chemokine, MCP-1, is required for autoimmune tissue injury in MRL-Faslpr mice. We now report that MRL-Faslpr mice genetically deficient in MCP-1 are partially protected from autoimmune disease, including injury to the kidney, lung, and skin and lymphadenopathy, resulting in a prolonged life span. Protection against tissue injury in MCP-1-deficient MRL-Faslpr mice results from a reduced infiltration of leukocytes (macrophages and T cells) toward parenchymal cells that no longer express MCP-1 and a diminution in cytokines known to promote tissue injury. Furthermore, we determined that the accumulation of these cells is a result of recruitment, but not proliferation, toward the parenchymal cells in proportion to MCP-1 expression. Thus, MCP-1 is responsible for recruiting macrophages and T cells into multiple tissues, including the kidney, lung, and lymph nodes, in MRL-Faslpr mice, which in turn results in autoimmune tissue destruction.

During inflammation, multiple parenchymal and leukocyte cell types express MCP-1 (36). Our challenge was to identify the cell types and locations expressing MCP-1 and determine the impact of cells producing MCP-1 on disease progression in MRL-Faslpr mice. We now report that MCP-1 is primarily expressed by parenchymal cells, and not infiltrating cells, in MRL-Faslpr mice, beginning before and then increasing during autoimmune disease. We determined that parenchymal epithelial cells express far more MCP-1 than other parenchymal cell types (endothelial cells, smooth muscle cells, and mesangial cells). Furthermore, we established that the accumulation of macrophages and T cells was directly proportional to MCP-1 expression by these parenchymal cells. This is consistent with our previous findings in a rapid, induced form of kidney damage, NSN, consisting of glomerular and tubular pathology (21). In NSN, we noted that MCP-1, abundantly expressed in TECs and barely detected in glomeruli, was responsible for tubular but not glomerular injury. We established that macrophages were recruited toward tubules expressing MCP-1 and after activation released molecules that induced TEC apoptosis (21). By comparison, the importance of MCP-1 in progressive autoimmune disease in MRL-Faslpr mice is broader than in NSN. First, MCP-1 is responsible for the accumulation of not only macrophages but also T cells within the kidney (tubules and glomeruli) and other tissues (lungs and lymph nodes) during autoimmune disease in MRL-Faslpr mice. For example, massively enlarged lymph nodes, composed of T cells, are characteristic of MCP-1–intact MRL-Faslpr mice (37). The most convincing evidence for MCP-1 fostering T cell accumulation is highlighted by the substantial decrease in the incidence of lymphadenopathy and lymph node size (>50% reduced at 8 mo of age) in MCP-1-deficient MRL-Faslpr mice. However, the numbers of T cells in the MCP-1-deficient MRL-Faslpr lymph nodes are not reduced to normal levels. In addition, MCP-1 is not required for splenomegaly in MRL-Faslpr spleens, as MCP-1–deficient MRL-Faslpr spleens were similar. Thus, MCP-1 is responsible in part for lymphadenopathy, but other molecules, perhaps chemokines, are required for splenomegaly in the MRL-Faslpr strain. Other candidates that are likely to be involved in splenomegaly include the β-chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β, reported to foster T cell trafficking (CD4, CD8) into lymph nodes after induced hypersensitivity (38).

In addition, we suggest that the broader impact of MCP-1 on kidney injury in the MRL-Faslpr mouse versus NSN is related to the progressive accumulation of MCP-1-dependent leukocytes over a longer period of disease manifestation in the MRL-Faslpr kidney and other tissues. Furthermore, comparison of MCP-1 immunostaining and mRNA levels in the two models indicates that tubules and glomeruli in MCP-1–intact MRL-Faslpr mice (5–6 mo) express higher amounts of MCP-1 than in NSN (7 d). Disease in NSN is mostly limited to the kidney, whereas MRL-Faslpr mice have multiple tissues targeted for destruction, each expressing MCP-1. Thus, by gene target-deleting MCP-1 in MRL-Faslpr mice, we determined that MCP-1 is responsible for recruiting macrophages and T cells to numerous tissues, each undergoing autoimmune disease.
The accumulation of macrophages and T cells surrounding parenchymal tissue in MRL-Faslpr mice results from recruitment and/or proliferation of these leukocytes (26, 27, 34, 35, 39). As MCP-1 induces IL-2 production by T cells (14), it is possible that MCP-1 promotes T cell proliferation in the kidney, lung, and lymph nodes. However, as few infiltrating cells are proliferating (PCNA+) at sites of MCP-1 expression within the kidney, lung, and enlarged lymph nodes in MRL-Faslpr mice, we conclude that the primary action of MCP-1 is to recruit macrophages and T cells into tissues and thereby promote autoimmune disease.

To further support the concept that MCP-1 initiates kidney injury via recruitment and not other immune events, we investigated whether MCP-1 caused an Ig isotype switch and alteration in Ig deposition in the kidney. Elevated Ig levels in MRL-Faslpr mice are dependent on T cell (40) and B cell (41, 42) events that are independent of MCP-1 in MRL-Faslpr mice. In addition, isotypes such as IgG3 compromise glomerular function (43). We did not detect any difference in the serum Ig isotypes nor in the amount or location of Igs in the kidney. It is interesting to note that despite the similarly high levels of serum and glomerular Igs, the glomeruli are better preserved functionally (loss of protein) and structurally in MCP-1-deficient versus MRL-Faslpr mice. This suggests that, when the Igs are deposited within the kidney, MCP-1 is required to attract leukocytes into the kidney to initiate glomerular and tubular/interstitial renal disease in MRL-Faslpr mice.

Protection from injury in MCP-1-deficient MRL-Faslpr kidneys is associated with reduced numbers of the nephritogenic cytokines (IFN-γ and CSF-1) and other chemokines in addition to MCP-1 that bind to the CCR2 receptor (MCP-3 and MCP-5). This could be either directly or indirectly related to the MCP-1 deletion in MRL-Faslpr mice. For example, MCP-1 immunomodulation of CD4 cells is related to the release of IFN-γ (14). We suggest that after MCP-1-dependent recruitment of leukocytes into the kidney, a cascade of events triggers the production of IFN-γ, CSF-1, MCP-3, and MCP-5, which each contribute to autoimmune kidney damage. For example, we have previously established that CSF-1, a macrophage growth factor, is responsible for promoting macrophage- and T cell-initiated kidney injury (35), whereas IFN-γ, with more complex actions, either thwarts (27) or fosters (25, 44) injury depending on when it is expressed during disease. In addition, MCP-3 and MCP-5 may be responsible for the migration of leukocytes into the kidney (45). We therefore speculate that the increase in MCP-1 is a proximal stimulus responsible for recruiting macrophages and T cells, which in turn are responsible for triggering the production of cytokines and other chemokines that lead to tissue injury.

Although MCP-1 depletion dampens injury in multiple tissues during autoimmune attack, these tissues are not totally protected. The kidneys, lungs, spleen, lymph nodes, and skin do not remain normal. The renal tubule/interstitium, glomeruli, lungs, and lymph nodes in MCP-1-deficient MRL-Faslpr mice are still invaded by leukocytes, although in far lower numbers than in MCP-1-intact MRL-Faslpr mice. Additionally, the limited number of MCP-1-deficient MRL-Faslpr mice that do not survive kidney glomerular and tubular disease and proteinuria is far lower than younger MCP-1-intact MRL-Faslpr mice that succumb. In contrast, vascular disease is not prevented in MCP-1-deficient kidneys and lungs. The accumulation of leukocytes around vessels, almost exclusively T cells, is just as abundant in MCP-1-deficient versus intact MRL-Faslpr kidneys and lungs. There are several possible explanations for this finding. Other chemokines may be responsible for recruiting these infiltrating leukocytes. For example, MCP-1 is weakly expressed in the vascular wall and perivascular leukocytes, whereas RANTES (regulated upon activation, normal T cell expressed and secreted) is abundant in both areas (data not shown). In addition, many perivascular leukocytes in the kidney and lung are proliferating (PCNA+) and, therefore, perivascular leukocytes may accumulate because of local proliferation. It is worth noting that perivascularis in the kidney contributes to mortality in wild-type MRL-Faslpr mice (45, 46) and, therefore, may be at least partially compromising in several tissues in MCP-1-deficient MRL-Faslpr mice. Finally, as the accumulation of leukocytes in the renal interstitium is focal in both MCP-1-intact and -deficient MRL-Faslpr mice, we suggest that molecules responsible for adhesion, cell activation, proliferation, and death, including integrins, selectins, and cytokines, may contribute to this process. Furthermore, we cannot rule out the possibility that other chemokines, in addition to MCP-1, enhance leukocytic recruitment. Thus, to achieve more complete protection from autoimmune disease in the MRL-Faslpr strain, we will have to identify additional therapeutic targets.

In conclusion, we suggest that MCP-1 is a therapeutic target to combat autoimmune/inflammatory diseases triggered by tissue leukocytic invasion. Our data further suggest that eliminating MCP-1 expression does not confer total protection. It is thus critical to identify the other therapeutic targets responsible for leukocyte invasion and expansion to confer total protection.

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References

1. Koh, D.R., A. Ho, A. Rahemtulla, W.P. Fung-Leung, H. Grieser, and T.W. Mak. 1995. M urine lupus in M R L/pmr mice lacking CD4 or CD8 T cells. Eur. J. Immunol. 25:2558–2562.

2. Tran, E.H., K. Hofstra, N. van Roon, C.D. Dijkstra, and T. Oomens. 1998. Immune invasion of the central nervous system parenchyma and experimental allergic encephalomyelitis, but not leukocyte extravasation from blood, are prevented in macrophage-depleted mice. J. Immunol. 161:3767–3775.

3. Rovin, B.H., M. Rumancik, L. Tan, and J. Dickerson. 1994. Glomerular expression of monocyte chemoattractant protein-1 in experimental and human glomerulonephritis. Lab. Invest. 71:536–542.

4. Wada, T., H. Yokoyama, S. Su, N. Mukaida, M. Iwano, K. Dohi, Y. Takahashi, T. Sasaki, K. Futuchi, C. Segawa, et al. 1996. Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. Kidney Int. 49:761–767.

5. Harigai, M., M. Hara, T. Yoshimura, E.J. Leonard, K. Inoue, and S. Kashiwazaki. 1993. Monocyte chemoattractant protein-1 (MCP-1) in inflammatory joint diseases and its involvement in the cytokine network of rheumatoid synovium. Clin. Immunol. Immunopathol. 69:83–91.

6. Zoja, C., X.H. Liu, R. Donadelli, M. Abbate, D. Testa, D. Corna, G. Taraboletti, A. Vecchi, Q.G. Dong, B.J. Rollow, et al. 1997. Renal expression of monocyte chemoattractant protein-1 in lupus autoimmune mice. J. Am. Soc. Nephrol. 8:720–729.

7. Berman, J.W., M.P. Guida, J. Warren, J. Amat, and C.F. Brossan. 1996. Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. J. Immunol. 156:3017–3023.

8. Valente, A.J., D.T. Graves, C.E. Vialle-Valentin, R. Delgado, and C.J. Schwartz. 1988. Purification of monocyte chemotactic factor secreted by nonhuman primate vascular cells in culture. Biochemistry. 27:4162–4168.

9. Carr, M.W., S.J. Roth, E. Luther, S.S. Rose, and T.A. Springer. 1994. Monocyte chemoattractant protein-1 acts as a T-lymphocyte chemoattractant. Proc. Natl. Acad. Sci. USA 91:3652–3656.

10. Allavena, P., G. Bianchi, D. Zhou, J. Van Damme, P. Jilek, S. Sozzani, and A. Mantovani. 1994. Induction of natural killer cell migration by monocyte chemotactic protein-1 and -3. Eur. J. Immunol. 24:3233–3236.

11. Jiang, Y., D.I. Bellr, G. Frendl, and D.T. Graves. 1992. Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. J. Immunol. 148:2423–2428.

12. Kim, J.J., L.K. Nottingham, J.I. Sin, A. Tsai, L. Morrison, J. Oh, K. Dang, Y. Hu, K. Kazahaya, M. Bennett, et al. 1998. CD8 positive T cells influence antigen-specific immune responses through the expression of chemokines. J. Clin. Investig. 102:1112–1124.

13. Higoboa, C.M., N.W. Lukacs, S.W. Chensue, R.M. Stret-
expansion in MRL-Fas<sup>−/−</sup> autoimmune interstitial nephritis: a negative regulatory pathway. J. Immunol. 160:4074–4081.

28. Lukacs, N.W., S.W. Chensue, R.E. Smith, R.M. Strieter, K. Warffingston, C. Wilke, and S.L. Kunkel. 1994. Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 alpha by inflammatory granuloma fibroblasts. Am. J. Pathol. 144:711–718.

29. Natori, Y., M. Sekiguchi, Z. Ou, and Y. Natori. 1997. Gene expression of CC chemokines in experimental crescentic glomerulonephritis (CGN). Clin. Exp. Immunol. 109:143–148.

30. Ji, J.G., J.A. Gonzaloz, C. Lloyd, L. Kremer, L. Lu, A.C. Martinez, B.K. Wershil, and J.C. Gutierrez-Ramos. 1996. Distinct expression and function of the novel mouse chemokine monocyte-chemoattractant protein-5 in lung allergic inflammation. J. Exp. Med. 184:1939–1951.

31. O'Connell, P.J., A. Pacheco-Silva, P.W. Nickerson, R.A. Muggia, M. Bastos, V.R. Kelley, and T.B. Strom. 1993. Unmodified pancreatic islet allograft rejection results in the preferential expression of certain T cell activation transcripts. J. Immunol. 150:1093–1104.

32. Ezure, T., T. Ishiwata, G. Asano, S. Tanaka, and K. Yokomura. 1997. Production of macrophage colony-stimulating factor by murine liver in vivo. Cytokine. 9:53–58.

33. Russell, E.S. 1968. The Jackson Laboratory's Pedigreed Expansion Stocks. Lifespan and Aging Patterns. Biology of the Laboratory Mouse. E.A. Green, editor. Dover Publications, Inc., New York. 512 pp.

34. Bloom, R.D., S. Florquin, and V.R. Kelley. 1993. Colony stimulating factor-1 in the induction of lupus nephritis. Kidney Int. 43:1000–1009.

35. Naito, T., H. Yokoyama, K.J. Moore, G. Dranoff, R.C. Mulligan, and V.R. Kelley. 1996. Macrophage growth factors introduced into the kidney initiate renal injury. Mol. Med. 2:297–312.

36. Rovin, B.H., and L.T. Phan. 1998. Chemotactic factors and renal inflammation. Am. J. Kidney Dis. 31:1065–1084.

37. Watanabe-Fukunaga, R., C.J. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Macrophage polarization disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature. 356:314–317.

38. Tedla, N., H.W. Wang, H.P. McNel, N. Di Girolamo, T. Hamartouzian, D. Wakefield, and A. Lloyd. 1998. Regulation of T lymphocyte trafficking into lymph nodes during an immune response by the chemokines macrophage inflammatory protein (MIP)-1 alpha and MIP-1 beta. J. Immunol. 161:5663–5672.

39. Moore, K.J., T. Wada, S.D. Barbee, and V.R. Kelley. 1998. Gene transfer of RANTES elicits autoimmune renal injury in MRL-Fas(lpr) mice. Kidney Int. 53:1631–1641.

40. Santoro, T.J., J.P. Portanova, and B.L. Kotzin. 1988. The contribution of L3T4<sup>+</sup> T cells to lymphoproliferation and autoantibody production in MRL-lpr/lpr mice. J. Exp. Med. 167:1713–1718.

41. Sobel, E.S., T. Katagiri, K. Katagiri, S.C. Morris, P.L. Cohen, and R.A. Eisenberg. 1991. An intrinsic B cell defect is required for the production of autoantibodies in the lpr model of murine systemic autoimmunity. J. Exp. Med. 173:1441–1449.

42. Merino, R., M. Iwamoto, L. Fossati, and S. Izui. 1993. Polyclonal B cell activation arises from different mechanisms in lupus-prone (NZB × NZW)F1 and MRL/Mp-j-lpr/lpr mice. J. Immunol. 151:6509–6516.

43. Izui, S., T. Berney, T. Shibata, and T. Fulpius. 1993. IgG3 cryoglobulins in autoimmune MRL-lpr/lpr mice: immunopathogenesis, therapeutic approaches and relevance to similar human diseases. Ann. Rheum. Dis. 52:S48–S54 (Suppl.).

44. Haaas, C., B. Ryffel, and M. Le Hir. 1997. IFN-γ is essential for the development of autoimmune glomerulonephritis in MRL-lpr/lpr mice. J. Immunol. 158:5484–5491.

45. Alexander, E.L., C.F. Moyer, G.S. Travlos, J.B. Roth and E.D. Murphy. 1985. Two histopathologic types of inflammatory vascular disease in MRL/Mp autoimmune mice. Model for human vasculitis in connective tissue disease. Arthritis Rheum. 28:1146–1155.

46. Bullard, D.C., B.D. King, M.J. Hicks, B. Dupont, A.L. Beaudet, and K.B. Elkon. 1997. Interleukin adhesion molecule-1 deficiency protects MRL/Mp-Fas<sup>−/−</sup> mice from early lethality. J. Immunol. 159:2058–2067.