Intercellular Adhesion Molecule-1 Deficiency Prolongs Survival and Protects against the Development of Pulmonary Inflammation During Murine Lupus

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Abstract

One of the characteristic features of the lupus syndrome in humans and mice is the organ-specific accumulation of leukocytes within a variety of different tissues; however, the etiology of this phenomenon remains unclear. The work presented here determined the role of intercellular adhesion molecule (ICAM)-1 in the development of pulmonary leukocyte accumulation by generating MRL/MpJ-*Fas*^{lpr} mice that are genetically deficient in this critical adhesion molecule. Interestingly, these MRL/MpJ-*Fas*^{lpr} ICAM-1 knockout mice exhibit prolonged survival times compared to littermates expressing ICAM-1. We have determined that lack of ICAM-1 completely abrogates the development of pulmonary inflammation but does not prevent the development of autoantibodies, lymphadenopathy, and glomerulonephritis. Furthermore, the lack of pulmonary inflammation was found to be due to decreased migration of leukocytes to the lung rather than decreased in situ proliferation of cells. (*J. Clin. Invest.* 1997. 100:963–971.) Key words: adhesion molecules • MRL/lpr • lung • life span • inflammation

Introduction

Systemic lupus erythematosus (SLE) is a multifactorial polygenic autoimmune disease which manifests as a diverse array of pathologies such as glomerulonephritis, vasculitis, and arthritis (1). Several murine models of this disease have contributed significantly to our understanding of the human disease, since most of the immunologic abnormalities exhibited in human lupus are also present in mouse models (2). In particular, the MRL/MpJ-*Fas*^{lpr} mouse spontaneously develops acute disseminated autoimmune disease which manifests as glomerulonephritis, vasculitis, and arthritis (3). Disease progression in these mice is characterized by hypergammaglobulinemia and autoantibody production as well as lymphoid hyperplasia (4, 5). This hyperplasia results in large accumulations of leukocytes within lymphoid and nonlymphoid tissues. Lymphoid infiltration in other organs is most prominent in the lung and in the kidneys (2, 6). The lpr defect is due to a mutation in the gene encoding for Fas, a cell surface apoptosis molecule that belongs to the TNF-receptor gene family (7). The characteristic lymphadenopathy and autoimmune disease which develop in lpr mice have been attributed to a Fas apoptosis defect in which activated T cells do not undergo activation-induced cell death (8). The importance of mutations in the *Fas* gene in human disease was highlighted recently by a report in which patients showed autoimmune lymphoproliferative disease in association with *Fas* gene defects (9). Although the hyperproliferative features of leukocytes in these mice seem to correlate with the Fas defect, the abnormal migration of these leukocytes to particular tissues and its connection to organ-specific autoimmunity are not understood. Therefore, the critical question as to the mechanism by which these leukocytes migrate to particular tissues remains unanswered.

Emigration of leukocytes from the circulation into surrounding tissues involves a series of events that have been described as (*a*) initial rolling of leukocytes on inflamed vascular endothelium; (*b*) activation of leukocytes by chemoattractants; (*c*) firm attachment to the blood vessel walls mediated by interaction between integrins and their ligands; and (*d*) transendothelial migration (10–12). Therefore, accumulation of leukocytes at a particular site may occur as a result of an abnormality arising at any of these steps in the interaction between leukocytes and vascular endothelium. Several studies have documented enhanced adhesion molecule expression in both leukocytes and endothelium during acute inflammation such as in allergic pulmonary disease (13) and nephritis (14). Vascular adhesion molecules are also upregulated during chronic inflammatory conditions, for example during autoimmune diseases such as arthritis, glomerulonephritis, diabetes, and thyroiditis (15). However, although activated vascular endothelium may contribute to inflammation by stimulating leukocyte migration, the precise mechanisms responsible for the accumulation of leukocytes into the lung or kidney during lupus remain unknown.

Intercellular adhesion molecule-1 (ICAM-1),[1] a ligand for the CD18 family of integrins, is normally expressed on several nonhematopoietic cell types and can be strongly upregulated

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1. *Abbreviations used in this paper:* dsDNA, double-stranded DNA; ICAM-1, intercellular adhesion molecule-1; ssDNA, single-stranded DNA.

on epithelial and endothelial cells by a variety of cytokines during inflammation (16). Although ICAM-1 is thought to function as an adhesion molecule that promotes leukocyte migration and extravasation, it may also act as a costimulatory molecule that under the right conditions results in T cell proliferation (17). Increased expression of ICAM-1 has been documented in several autoimmune diseases in humans and mice, including SLE (15). In particular, ICAM-1 expression is augmented in mesangial and tubular epithelial cells in lupus nephritis, and has been suggested to be a possible indicator of disease severity (18). Similarly, kidneys from MRL/MpJ-*Fas*^{lpr} mice show enhanced expression of ICAM-1 within the glomerular mesangium, the endothelium of large vessels, and in the brush border of proximal tubules (19). ICAM-1 is also upregulated on both the pulmonary epithelium and endothelium during models of lung inflammatory disease (20, 21). Moreover, anti-ICAM-1 antibodies inhibit leukocyte infiltration and ameliorate tissue injury during both lung inflammatory disease (22, 23) and autoimmune renal disease (24).

The work presented here has determined the role of ICAM-1 in the development of lung and renal leukocyte accumulation by producing MRL/MpJ-*Fas*^{lpr} mice deficient in this critical adhesion molecule. We have determined that lack of ICAM-1 completely abrogates the development of pulmonary inflammation but does not prevent the development of autoantibodies, lymphadenopathy, and glomerulonephritis. Furthermore, the lack of pulmonary inflammation was found to be due to decreased migration of leukocytes to the lung.

Methods

Generation of ICAM-1–deficient MRL/MpJ-Fas^{lpr} *mice.* ICAM-1–deficient mice were generated by homologous recombination (25). The mutated alleles for ICAM-1 were then introduced into the MRL/lpr background by backcrossing ICAM-1 knockout mice with MRL/MpJ-*Fas*^{lpr} mice (The Jackson Laboratory, Bar Harbor, ME). Tail DNA from offspring was obtained at each generation, and Southern blots were used to determine the genotype of the progeny. The work described here results from analysis of mice in the eighth generation of backcrossing. Heterozygous mice were obtained when the eighth intercrossed with their siblings to produce MRL/MpJ-*Fas*^{lpr} ICAM-1 +/+ and MRL/MpJ-*Fas*^{lpr} ICAM-1 −/− mice referred to throughout this manuscript as lpr/ICAM-1+/+ and lpr/ICAM-1−/− mice, respectively. These two strains are the control and experimental strains of mice that are the subjects of this study. The denomination MRL/MpJ-*Fas*^{lpr} in this manuscript identifies exclusively the parental strain used for the backcrossing and as additional controls in some experiments.

Survival analysis. Kaplan-Meier estimates of survival probabilities were computed for both types of mice using the LIFETEST procedure in SAS. The Wilcoxon test statistic was used to assess the association between genotype and survival time and whether its effect varied by gender. A Cox proportional hazards model was used to test the significance of genotype and gender as predictors of survival time, using the PHREG procedure in SAS.

Assessment of lymphoid hyperplasia. To determine the effect of ICAM-1 on the development of the characteristic generalized lymphadenopathy observed in MRL/MpJ-*Fas*^{lpr} mice, the cellularity of spleen and lymph nodes was assessed at intervals during the life span of mice. Spleen and lymph nodes (cervical, axial and mesenteric) were excised, and cell suspensions were prepared and counted.

Production of autoantibodies. Mice were bled from the tail vein at intervals from 6 to 18 wk. Levels of IgG or IgM antibodies reactive with either single-stranded (ss) or double-stranded (ds)DNA were determined in an ELISA (26). For each sample, a semilog curve of serum dilution versus OD was plotted to obtain a midpoint titer. Reactivity of IgG or IgM antibodies with ss- or dsDNA was compared to that in 30-wk-old MRL/MpJ-*Fas*^{lpr} mice.

Histological assessment of organs. Kidneys and lungs were excised from mice killed at intervals throughout disease, and fixed in 10% formaldehyde in PBS, embedded in paraffin (Tissue-Tek; Miles Inc., Somerset, MA), and sectioned at 3 μm. Morphological analysis was performed on sections stained with hematoxylin and eosin. Further, quantitative analysis was performed specifically on lung samples in order to compare both the extent and size of infiltration in lpr/ICAM-1+/+ and lpr/ICAM-1−/− mice. First, 100-μm-diameter vessels were screened for the presence of a cellular infiltrate of at least three cells deep at a magnification of 400, with at least 100 vessels counted per section. To determine the sizes of pulmonary infiltrates in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice, the area of lung tissue covered by infiltrate was calculated in sections at low power (at a magnification of 100) using NIH Image 1.56. At least six fields of at least 0.01 mm^2 were scanned, and the mean percentage area was determined for each mouse of each genotype.

Immunohistochemical determination of cellular proliferation. The in vivo incorporation of BrdU (Sigma Chemical Co., St. Louis, MO) was determined to assess the extent of cellular proliferation within lungs and kidneys (27). Mice were injected intraperitoneally with 100 mg/kg BrdU (Sigma Chemical Co.) 90 min before killing and tissues were removed, fixed in 10% formaldehyde in PBS, and embedded in paraffin. 4-μm sections were digested in 0.1% trypsin and then 1 M hydrochloric acid. Sections were then stained for BrdU using an avidin biotin staining method. Briefly, incorporated BrdU was detected by overnight incubation with a biotin-labeled monoclonal mouse anti-BrdU antibody (Caltag Labs., South San Francisco, CA), followed by streptavidin peroxidase (DAKO Corp., Carpinteria, CA) for 1 h, and then with peroxidase substrate solution (20 mg diaminobenzidine in 10 ml PBS containing 0.01% hydrogen peroxide) for 10 min.

To compare proliferation between groups of mice, sections were analyzed microscopically, and the percentage of positively stained cells was counted in perivascular areas in each section, with at least 500 cells counted per section. The fact that very few infiltrating cells were observed in lpr/ICAM-1−/− mice necessitated counting cells from a much higher number of vessels compared to lpr/ICAM-1+/+ mice, where 500 cells were generally counted around only one or two vessels, thus introducing some bias in field selection in lpr/ICAM-1−/− mice but not in lpr/ICAM-1+/+ mice.

In vivo lymphocyte migration assay. Leukocyte suspensions were prepared from cervical lymph nodes of 12-wk-old lpr/ICAM-1+/+ and lpr/ICAM-1−/− mice, labeled with the fluorochrome PKH-26 (Sigma Chemical Co.) (28), and injected intravenously into siblings of comparable genotype, sex, and age (10^7 cells per mouse). Mice were killed after 24 or 96 h, and lungs were excised, inflated with OCT compound (Cryoform, IEC, Needham Heights, MA), and frozen. These particular time points were chosen to avoid the possibility of observing solely the nonspecific sticking of cells within the lung vasculature that may occur after intravenous injection. Sections from each recipient mouse were fixed with 4% paraformaldehyde and mounted in Fluoromount (Southern Biotechnology Associates, Inc., Birmingham, AL). Labeling efficiency was calculated in cell smears prepared before injection, and in each sample > 97% of the cells were found to be labeled. Sections were examined under fluorescence illumination at a magnification of 400, and PKH-positive cells were enumerated within 15 randomly chosen fields per section, in duplicate sections per mouse. Cells were observed in lpr/ICAM-1+/+ mice (and to a lesser extent in lpr/ICAM-1−/− mice) not in blood vessels but rather within the lung parenchyma, both in perivascular and peribronchiolar areas and in alveolar parenchyma.

Assessment of 24-h proteinuria. Urine protein excretion was measured in timed overnight specimens collected from individual mice kept in metabolism cages and assayed by the sulphosalicylic acid method (29).

Results

ICAM-1 deficiency results in improved survival. Mutant alleles for ICAM-1 were introduced to the MRL/lpr background by backcrossing ICAM-1–deficient mice with MRL/MpJ-*Fas*^{lpr} mice for eight consecutive generations. ICAM-1–deficient mice were generated previously by gene targeting in embryonic stem cells (25), and have been shown to be protected from a variety of inflammatory diseases, including septic shock, ischemia reperfusion injury, and arthritis (25, 30, 31). The lpr-ICAM-1+/+ mice generated showed life spans comparable to those expected for MRL/MpJ-*Fas*^{lpr} mice (3). ICAM-1 deficiency in MRL/MpJ-*Fas*^{lpr} mice conferred an advantage in survival over lpr-ICAM-1+/+ mice, with the mean survival time increasing from 20.9±4.0 to 43.0±13.1 wk for female, and from 22.9±5.1 to 37.0±12.5 wk for male mice. The difference in survival times was found to be highly significant for both sexes ($P <$ 0.0001, Wilcoxon test). Proportional hazards analysis revealed that lpr-ICAM-1+/+ mice were more likely to die than lpr-ICAM-1−/− mice, and that the risk of death may be influenced by the interaction between genotype and gender. Female lpr-ICAM-1+/+ mice were 20 times more likely to die (95% confidence interval 5.9, 67.8) than female lpr-ICAM-1−/− mice (Fig. 1 *A*), while male lpr-ICAM-1+/+ mice were 4.6 times more likely to die (95% confidence interval 1.7, 12.5) than male lpr-ICAM-1−/− mice (Fig. 1 *B*). However, in this model, gender was only borderline statistically significant (c2 = 4.96, 2d.f. $P = 0.08$), possibly due to the relatively small sample size for this type of analysis.

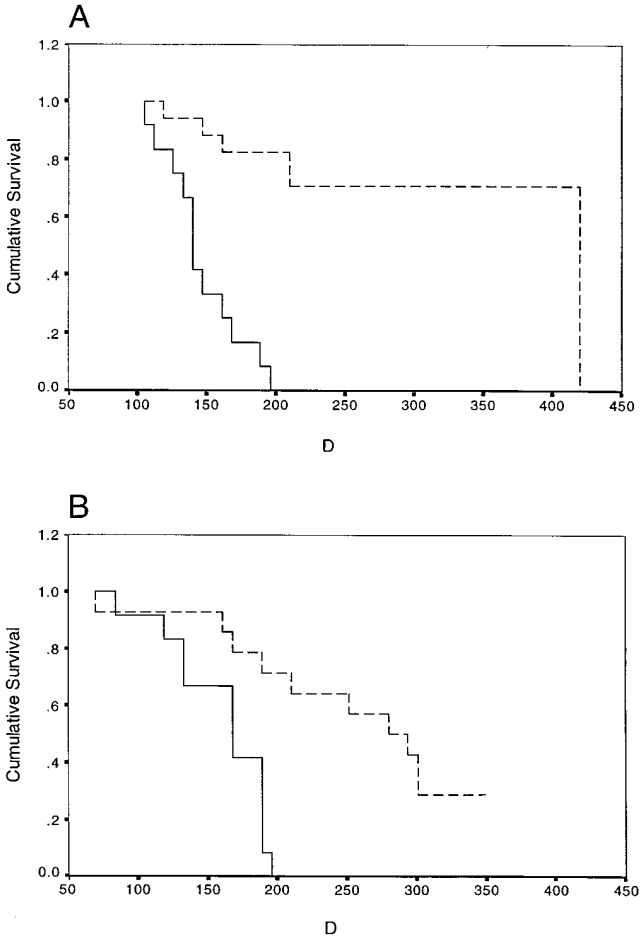

Figure 1. ICAM-1 deficiency results in improved survival for MRL/MpJ-*Fas*^{lpr} mice. Survival analysis of the life span of (*A*) female and (*B*) male lpr-ICAM-1+/+ (*solid line*, $n = 24$) and lpr-ICAM-1−/− mice (*dotted line*, $n = 32$) using the LIFETEST procedure. The difference in survival between genotypes is highly significant, with $P <$ 0.0001 (Wilcoxon test).

Development of characteristic lupus phenotype. Since lupus is a polygenic disease, it is important to ensure that incorporation of the fraction (< 0.4% by the eighth generation) of the 129SV×C57Bl/6 genome carried with the ICAM-1 locus does not interfere with the development of the MRL/MpJ-*Fas*^{lpr} phenotype, independent of the ICAM-1 deficiency. Therefore, careful analysis of lpr-ICAM-1+/+ mice was performed to determine that these mice developed the standard features of the MRL/MpJ-*Fas*^{lpr} phenotype. A characteristic hallmark of MRL/MpJ-*Fas*^{lpr} mice is profound hyperplasia of the spleen and lymph nodes (5). Since interactions mediated by ICAM-1 with its ligand(s) could be involved in both migration and proliferation of lymphocytes, the size and cellularity of spleens and three different sets of lymph nodes (cervical, axial, and mesenteric) were compared. ICAM-1 deficiency did not significantly affect the typical enlargement of spleen or any of the three sets of lymph nodes at any stage throughout disease development. Fig. 2 *A* illustrates the lack of difference in cellularities of spleens and cervical nodes from lpr-ICAM-1−/− and lpr-ICAM-1+/+ mice. Similarly, the cellularities of axial and mesenteric nodes were not significantly different in the presence or absence of ICAM-1 in their genome [7.3±3.9 vs. 5.3±2.2 and 10.2±4.8 vs. 11.3±6.4 million cells/node ($n = 7$ mice), respectively, at 11 wk]. There was no distinguishable difference in proportions of leukocyte subsets between groups of mice in any of the sets of lymph nodes or spleens, including the B220$^+$CD4$^-$CD8$^-$ T cell population that is expanded in MRL/MpJ-*Fas*^{lpr} mice (data not shown). The increase in spleen and lymph node size observed in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice was found to be comparable to that characteristic of similarly aged MRL/MpJ-*Fas*^{lpr} mice from the parental strain (Fig. 2 *A*). The typical destruction of thymic architecture that occurs in mice of the parental MRL/MpJ-*Fas*^{lpr} strain also developed in the lpr-ICAM-1 mice reported here, regardless of the presence or absence of ICAM-1 in their genome (data not shown).

Similarly, the lack of ICAM-1 did not influence the production of autoantibodies, as lpr-ICAM-1−/− mice developed antibodies reactive with ssDNA (data not shown) or dsDNA (Fig. 2 *B*). The profile of antibody production was comparable between mice, and by 12 wk both groups produced levels of DNA-binding antibodies similar to these in wild-type MRL/MpJ-*Fas*^{lpr} mice. This finding was observed for antibodies reactive with either ds- or ssDNA of IgM (data not shown) and IgG (Fig. 2 *B* and data not shown) isotypes. Both groups of lpr mice (ICAM-1+/+ and ICAM-1−/−) also produced rheumatoid factor at later stages of disease at similar levels to those found in mice from the MRL/MpJ-*Fas*^{lpr} parental strain (data not shown).

ICAM-1 deficiency protects against pulmonary inflammation. The most striking difference between lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice was in the integrity of lung architecture. lpr-ICAM-1+/+ mice developed widespread perivascular and peribronchiolar mononuclear infiltrates by 12 wk of

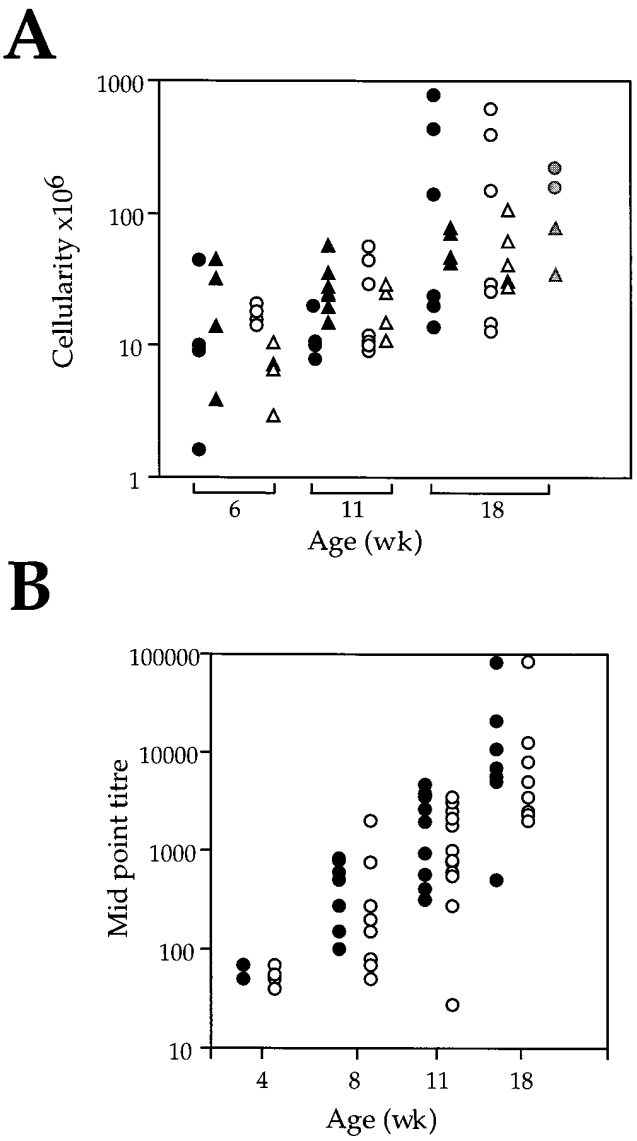

Figure 2. The characteristic MRL/MpJ-*Fas*^{lpr} phenotype develops regardless of the presence or absence of ICAM-1. (*A*) Cellularity of lymphoid tissue. Cells were counted in suspensions made from spleens (*circles*) and cervical lymph nodes (*triangles*) from lpr-ICAM-1+/+ (*filled symbols*) or lpr-ICAM-1−/− mice (*open symbols*) killed at different time points of disease, with four mice per group at six wk and seven mice per group at other time points. The lymphoid tissue enlargement was comparable to that in age-matched mice of the parental MRL/MpJ-*Fas*^{lpr} strain (*gray symbols*). (*B*) Autoantibody production. IgG antibodies reactive with dsDNA were measured by ELISA in serial tail bleeds with between 5 and 15 mice per group.

age (Fig. 3, *A* and *B*). In contrast, the pulmonary architecture in lpr-ICAM-1−/− mice was normal, with mononuclear cells rarely present around larger vessels (Fig 3, *C* and *D*) and never around the airways. The infiltrates in lung tissue of lpr-ICAM-1+/+ mice were composed of ~ 50% T cells and 50% macrophages/monocytes, as determined by immunohistochemical phenotyping using antibodies specific for T cells (using Thy1) and mononuclear phagocytes (using MOMA-2) (data not shown), confirming previous descriptions (3, 32). There were scant numbers of neutrophils, and the infiltrate was generally confined to vessels near the larger airways. This phenomenon was not seen in younger mice (6 wk old) of either genotype, but was present in the majority of lpr-ICAM-1+/+ mice by 11 weeks (as well as in mice of the parental MRL/MpJ-*Fas*^{lpr} strain). Quantitative histological analysis revealed that lpr-ICAM-1+/+ mice not only had greater numbers of infiltrates within their lungs, but also that infiltrates were of a significantly larger size. At 11 wk, > 60% of the vessels were surrounded by mononuclear infiltrates in the majority of the lpr-ICAM-1+/+ mice, a significantly higher proportion than in lpr-ICAM-1−/− mice ($P < 0.05$, Mann-Whitney Wilcoxon test) (Fig. 3 *E*). In addition, pulmonary infiltrates were consistently larger in lpr-ICAM-1+/+ mice than in lpr-ICAM-1−/− mice; thus, a larger area was occupied by infiltrating cells ($P < 0.005$, Mann-Whitney Wilcoxon test) (Fig. 3 *F*).

Role of ICAM-1 in migration and proliferation during pulmonary inflammation in lpr-ICAM-1 mice. ICAM-1 is thought to function as a key molecule in leukocyte migration as well as a costimulatory molecule to promote cell proliferation, and as such the lack of inflammatory infiltrate in the lungs of lpr-ICAM-1–deficient mice reflects the importance of ICAM-1 in these processes during lupus. To distinguish between these two processes during the development of pulmonary inflammation in MRL/MpJ-*Fas*^{lpr} mice, the capacity of leukocytes to migrate or proliferate was examined in vivo. First, the potential of leukocytes to migrate to the lungs of lpr-ICAM-1+/+ or lpr-ICAM-1−/− mice was assessed. Fluorescent-labeled lpr-ICAM-1+/+ or lpr-ICAM-1−/− lymph node cells were injected into age-, sex- and genotype-matched recipients, and the lungs were excised 24 or 96 h later. Lung sections from these two groups of mice were analyzed to determine the number of fluorescent cells present in 15 randomly chosen fields at high power. Many more leukocytes migrated to the lungs of 12-wk-old lpr-ICAM-1+/+ mice than in lpr-ICAM-1−/− mice at both 24 and 96 h after injection (Fig. 4 *A*), and this difference was found to be highly significant ($P < 0.05$, Mann-Whitney Wilcoxon test).

To investigate the possibility that infiltrates arise in the lungs of MRL/MpJ-*Fas*^{lpr} mice as a result of in situ proliferation of inflammatory leukocytes, we assessed the comparative numbers of these cells proliferating within the tissues of both lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice. We found that lack of ICAM-1 does not overtly affect the proliferative capacity of infiltrating leukocytes in the lung during disease, as shown in Fig. 4 *B*. In vivo labeling of cells with the proliferation marker BrdU revealed that ~ 50% of the cells within pulmonary infiltrates were proliferating at the time of killing of either 12-wk-old lpr-ICAM-1+/+ or lpr-ICAM-1−/− mice, with no difference in numbers discernible between groups of mice. It is important to stress that in order to make an estimate of the numbers of proliferating cells within the lungs of lpr-ICAM-1−/− mice, it was necessary to select those vessels which were surrounded by some cells—requiring examination of at least three large vessels. This indicates some bias in the analysis, since only one vessel per field was sufficient to determine proliferation in 500 cells from lpr-ICAM-1+/+ mice.

Glomerulonephritis. Glomerulonephritis is a hallmark of disease in lupus-prone mice (2); therefore, to ascertain whether the abrogation of pulmonary infiltration was due to a generalized impairment in leukocyte migration to tissues, the development of renal disease was examined in lpr-ICAM-1+/+

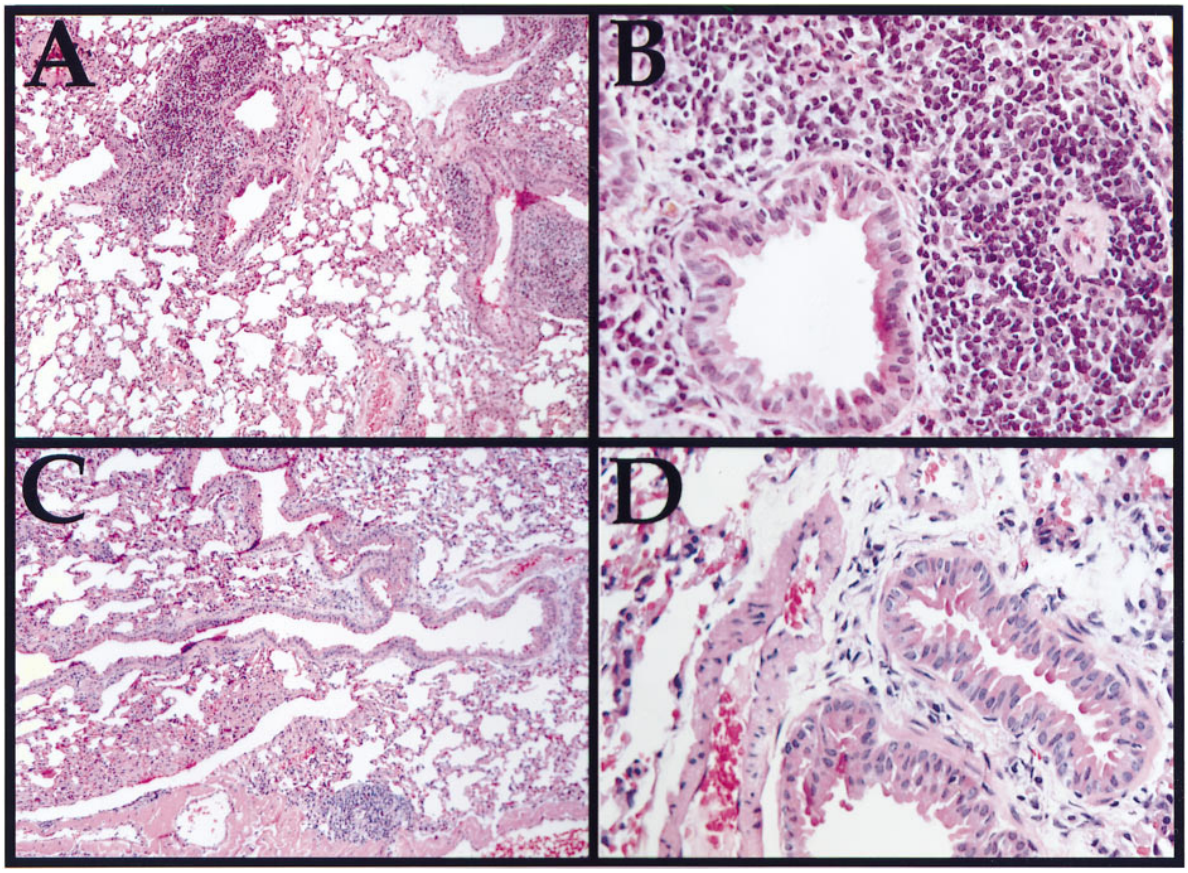

Figure 3. ICAM-1 deficiency protects against pulmonary inflammation in MRL/MpJ-Fas^{lpr} mice. (*A*) Hematoxylin and eosin staining of 4-μm sections from a representative lpr-ICAM-1+/+ mouse (*A* and *B*) displaying prominent peribronchiolar and perialveolar mononuclear infiltrates which were only occasionally seen in lpr-ICAM-1−/− mice (*C* and *D*). Original magnification is 100 for *A* and *C*, 400 for *B* and *D*. (*E*) Quantitative estimate of vessels with infiltrates in lpr-ICAM-1+/+ (*filled circles*) and lpr-ICAM-1−/− mice (*open circles*). 100 vessels (of at least 100 μm in diameter) were assessed for the presence of an inflammatory infiltrate of at least three cells deep, and percentages were calculated. (*F*) Comparison of pulmonary infiltrate size in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice. The average area of lung tissue covered by infiltrates was calculated in sections at low power (×100) using NIH Image 1.56. At least six fields of 0.01 mm^2 were scanned for each mouse, and the mean percentage area is shown for six mice of each genotype. The significance of differences in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice was estimated using the Mann-Whitney Wilcoxon test.

and lpr-ICAM-1−/− mice. Both groups of mice exhibited a diffuse proliferative nephritis that was variable among mice within groups. However, all mice showed some degree of renal abnormality, including perivascular accumulation of leukocytes (Fig. 5, *A* and *B*), interstitial and glomerular infiltrates, capillary loop occlusion, and mesangial matrix expansion (Fig. 5 *C*). Immunohistochemistry with specific antibodies determined that leukocytic infiltrates were found to be composed of ~ 50% T cells and 50% mononuclear phagocytes in both groups of mice (data not shown). A comparable degree of proliferation was observed within infiltrates in both groups of mice (data not shown). In addition, levels of 24-h proteinuria were indistinguishable between groups of mice at 11 and 18 wk of disease.

Discussion

This work has examined the role of ICAM-1 in the development and pathogenesis of autoimmune disease occurring in MRL/MpJ-*Fas*^{lpr} mice by producing MRL/MpJ-*Fas*^{lpr} mice that are genetically deficient in this adhesion molecule. We have shown that, although ICAM-1 is not required for the development of the typical serological and lymphoid abnormalities associated with disease progression in these mice, an in-

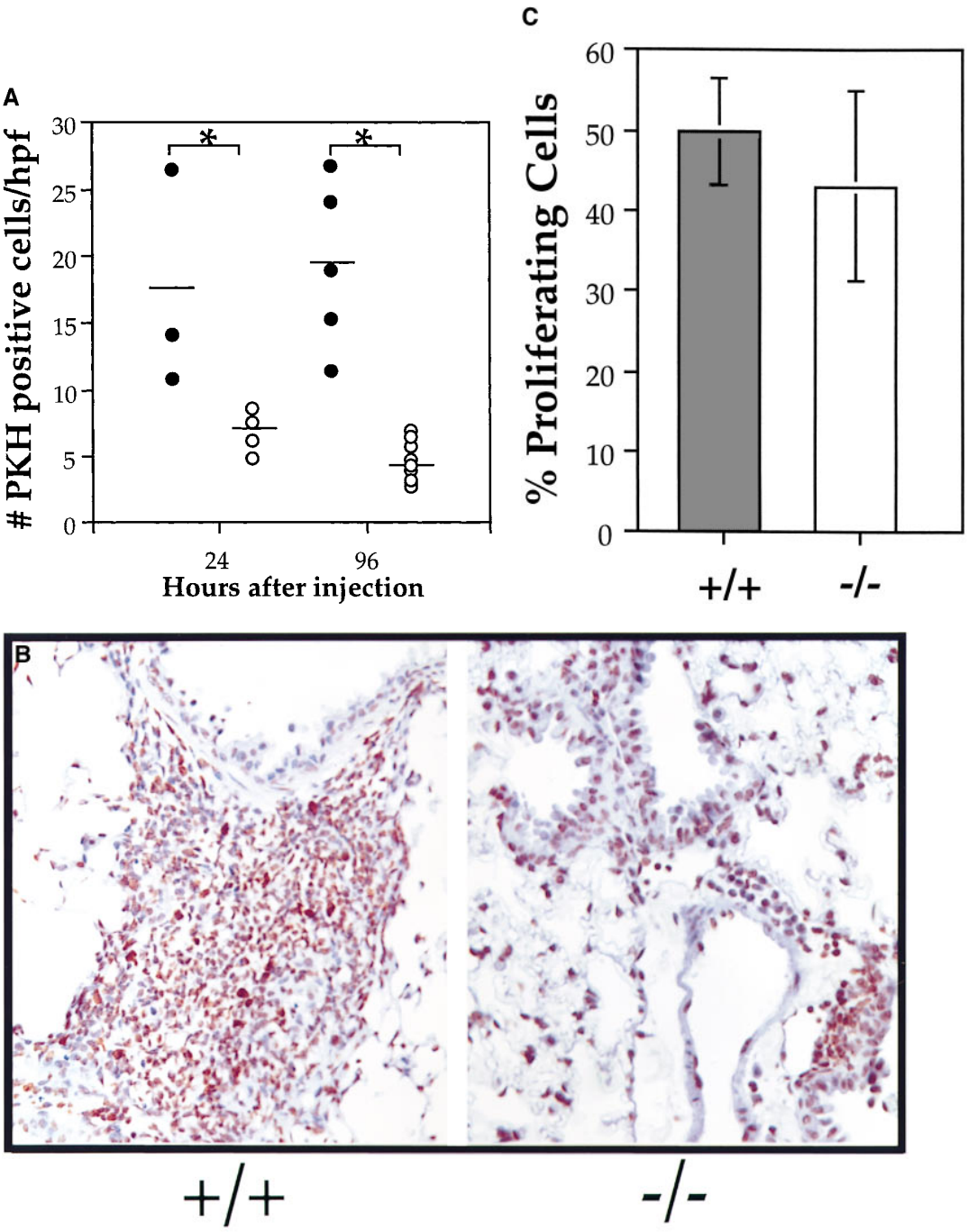

Figure 4. Role of ICAM-1 in migration and proliferation during pulmonary inflammation in lpr-ICAM-1 mice. (*A*) Lymphocyte migration to the lungs of 11-wk-old lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice in vivo. Leukocytes were isolated from cervical lymph nodes of 11-wk-old lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice, labeled with the fluorochrome PKH-26, and injected intravenously into age-, sex- and genotype-matched littermates. Mice were then killed 24 or 96 h later, and the lungs were excised and sectioned. Positively stained cells were quantified in 15 randomly chosen fields, in duplicate sections at a magnification of 400. Significance of differences between lpr-ICAM-1+/+ (*filled circles*) and lpr-ICAM-1−/− mice (*open circles*) was calculated using the Mann-Whitney Wilcoxon test. (*B*) Cell proliferation in the lungs of 11-wk-old lpr-ICAM-1+/+ (*left*) and lpr-ICAM-1−/− mice (*right*) injected with BrdU before killing and then stained with a specific mAb. Original magnification is 400. (*C*) Proliferation of cells in lungs from each group of mice was obtained by counting the proportion of positively stained cells within perivascular infiltrates, with at least 500 cells counted per section. Fields were selected at random within sections from lpr-ICAM-1+/+ mice, but the selection in lpr-ICAM-1−/− mice was biased towards the few areas containing cells.

nate deficiency in ICAM-1 confers a significant survival advantage to MRL/MpJ-*Fas*^{lpr} mice. Moreover, ICAM-1 is critically involved in the development of pulmonary but not renal inflammation.

In this study, the most striking difference between groups of mice was the increase in survival conferred on MRL/MpJ-*Fas*^{lpr} mice deficient in ICAM-1. The average survival time for MRL/MpJ-*Fas*^{lpr} mice is ~ 20 wk (3), and we found consistently that lpr-ICAM-1+/+ mice obtained by intercrossing mice that were heterozygous mutants for ICAM-1 after eight generations of backcrossing with MRL/MpJ-*Fas*^{lpr} mice show comparable life spans. However, we found that lack of ICAM-1 doubled the life span in lpr-ICAM-1−/− mice. The usual cause of death for mice of this strain is thought to be the profound glomerulonephritis that is characteristic of the disease in both mice and humans (3, 33). However, we found that although renal pathology was comparable in lpr-ICAM-1−/− and lpr-ICAM-1+/+ mice at all stages of disease, pulmonary inflammation was abrogated in the former. Thus, there seems to be an association between increased life span and lack of pulmonary inflammation, although we cannot determine whether lpr-ICAM-1+/+ mice died as a result of lung or renal

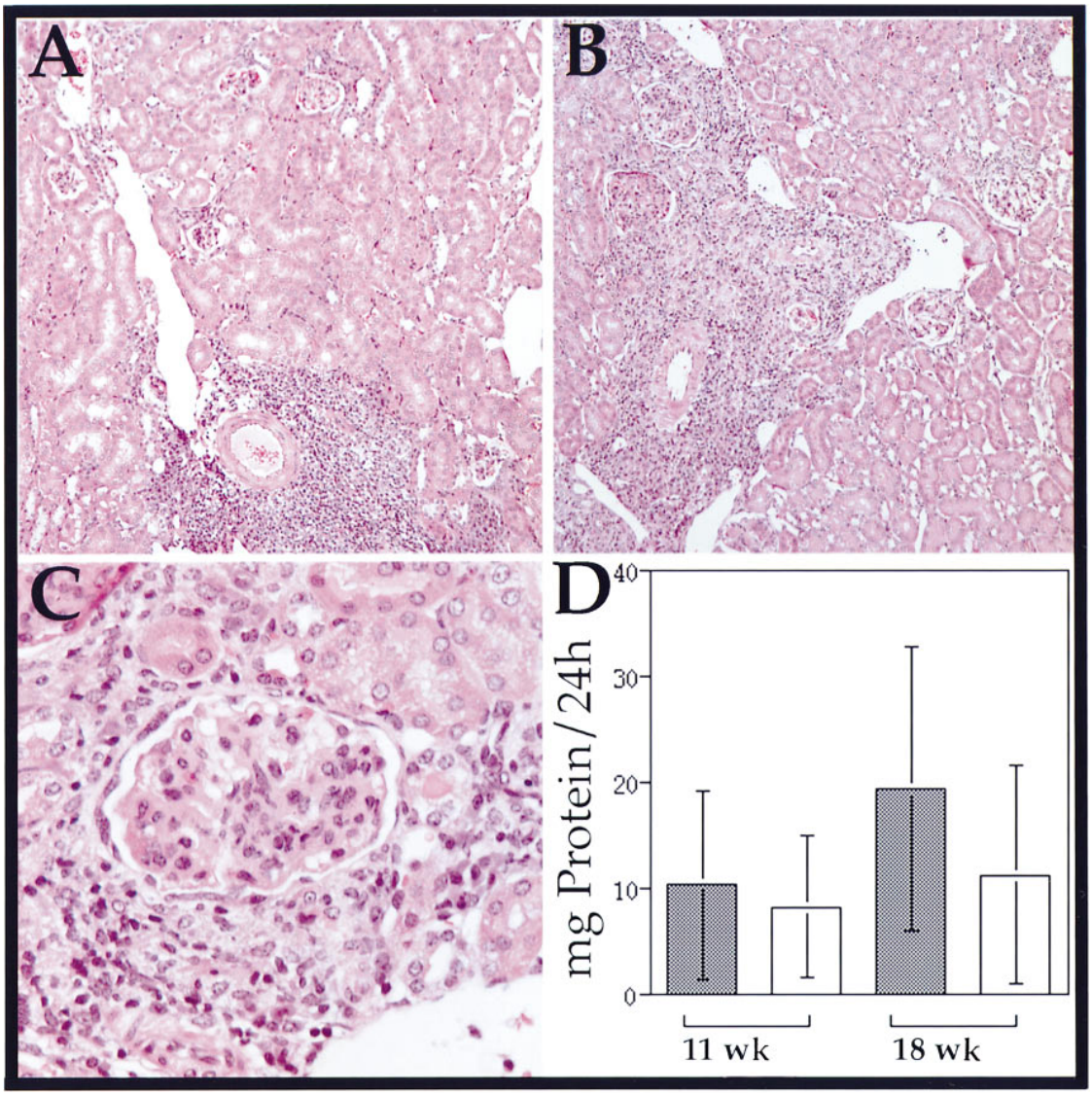

Figure 5. Glomerulonephritis is comparable in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice. Hematoxylin and eosin staining of kidney tissue from 11-wk-old lpr-ICAM-1+/+ (*A*) and lpr-ICAM-1−/− mice (*B* and *C*). Both genotypes showed extensive perivascular and interstitial mononuclear infiltrates (*A* and *B*) in conjunction with mesangial hypercellularity and thickening (*C*). Original magnification is 100 for *A* and *B*, 400 for *C*. (*D*) Renal dysfunction lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice throughout the course of disease. No difference in levels of 24-h proteinuria was discerned in lpr-ICAM-1+/+ (*filled bars*) and lpr-ICAM-1−/− mice (*open bars*) analyzed at 11 or 18 wk of disease.

pathology. Nevertheless, degree of pulmonary inflammation may be an unanticipated factor influencing the life span of lupus-prone mice.

Pulmonary inflammation is a common feature of lupus in humans (34) and mice (3). Lymphoid infiltration in MRL/MpJ-*Fas*^{lpr} mice has been found previously to be most prominent in the lung, with cells aggregating in the peribronchiovascular and hilar regions (6). Similarly, we observed that mononuclear infiltrates were widespread in the lungs of lpr-ICAM-1+/+ mice from 8 wk of age. However, this phenomenon was routinely absent in MRL/MpJ-*Fas*^{lpr} mice deficient in ICAM-1, suggesting that ICAM-1 is critical for accumulation of leukocytes in the lung. ICAM-1 has been found previously to be a key molecule in the recruitment of T cells to the lung during allergic pulmonary disease (13, 21). ICAM-1 is expressed by the vascular endothelium and the bronchiolar epithelium as well as by lymphocytes, and is thought to play a vital role in both the vascular and alveolar compartments in the initiation of lung inflammatory injury (23).

Leukocytes can potentially accumulate in tissues as a result of increased migration of inflammatory cells or by proliferation of leukocytes. The results presented here indicate that the defect in development of pulmonary inflammation in lpr-ICAM-1−/− mice is at the level of migration rather than in proliferation. However, we cannot formally exclude that small differences in the proliferation rates of leukocytes in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice could contribute to the markedly impaired migration of leukocytes to lungs of lpr-ICAM-1−/− mice and thus contribute to the final phenotype observed.

The lack of inflammatory infiltrates in ICAM-1–deficient MRL/MpJ-*Fas*^{lpr} mice was unique to the lung, since the degree of lymphadenopathy and the glomerular and interstitial infiltration in the kidneys was comparable in both groups of mice. Although ICAM-1 has been found to be upregulated in lupus nephritis in mice (19), it does not seem to be absolutely required for leukocyte infiltration of kidneys. This may be due to the equivalent levels of autoantibodies in serum of lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice. It is also possible that other adhesion molecules compensate in the absence of ICAM-1. The VCAM-1/VLA-4 as well as the ICAM-1/LFA-1 interactions have been highlighted as providing the predominant adhesive interactions at inflammatory sites during SLE (35). Based upon these results, we propose that pulmonary and renal inflammation arise during the same disease in this model by two different and distinct mechanisms. It has been suggested that humoral immunity is the critical trigger for induction of nephritis during lupus (36), and we found that autoantibody levels are comparable in lpr-ICAM-1+/+ and lpr-ICAM-1−/− mice throughout the duration of disease. Autoantibodies may induce renal inflammation by forming in situ immune complexes (37) or by cross-reacting with glomerular structures (38). Deposition of immune complexes may cause macrophage activation and thus the secretion of proinflammatory cytokines such as TNF, IFNγ, and IL-1, which may in turn induce expression of vascular adhesion molecule-1 on vascular endothelium—particularly in glomeruli (35). In contrast, pulmonary inflammation seems to occur independently of the influence of autoantibodies and seems to be related primarily with the ICAM-1–dependent accumulation of leukocytes in the lung.

This study has shown that ICAM-1 is critically involved in the development of pulmonary inflammation in MRL/MpJ-*Fas*^{lpr} mice, and that ICAM-1 deficiency prolongs the survival of these mice. Involvement of the lung in SLE may be as common as renal involvement and more common than involvement of the central nervous system (39). Most studies indicate that pulmonary involvement occurs > 50% of the time in SLE (34), with autopsy series reporting an even greater frequency of involvement (40). The results of this study indicate that the role of ICAM-1 in pulmonary inflammation in patients with SLE merits further study.

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References

1. Tan, E.M., A.S. Cohen, and J.F. Fries. 1982. The 1982 revised criteria for the classification of systemic erythematosus. *Arthritis Rheum.* 25:1271–1277.

2. Theofilopoulos, A.N. 1992. Murine models of lupus. *In* Systemic Lupus Erythematosus. R.G. Lahita, editor. Churchill Livingstone, New York. 121–169.

3. Theofilopoulos, A.N., and F.J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269–390.

4. Tan, E. 1982. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv. Immunol.* 33:167–189.

5. Dixon, F.J. 1981. Murine systemic lupus erythematosus. *Immunol. Today.* 2:245–257.

6. Sunderrajan, E.V., W.N. McKenzie, T.R. Lieske, J.L. Kavanaugh, S.R. Braun, and S.E. Walker. 1986. Pulmonary inflammation in autoimmune MRL/Mp-lpr/lpr mice. Histopathology and bronchoalveolar lavage evaluation. *Am. J. Pathol.* 124:353–362.

7. Watanabe-Fukunaga, R., C. Brannan, N. Copeland, N. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature (Lond.).* 356:314–317.

8. Russel, J.H., B. Rush, C. Weaver, and R. Wang. 1993. Mature T-cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. *Proc. Natl. Acad. Sci. USA.* 90:4409–4413.

9. Sneller, M.C., J. Wang, J.K. Dale, W. Strober, L.A. Middleton, Y. Choi, T.A. Fleischer, M.S. Lim, E.S. Jaffe, J.M. Puck, et al. 1977. Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal apoptosis. *Blood.* 89:1341–1348.

10. Springer, T.A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 76:301–314.

11. Butcher, E.C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell.* 67:1033–1036.

12. Lasky, L.A. 1992. Selectins: interpreters of cell-specific carbohydrate information during inflammation. *Science (Wash. DC).* 258:964–969.

13. Wegner, C., R. Gundel, P. Reily, N. Haynes, L. Letts, and R. Rothlein. 1990. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science (Wash. DC).* 247:456–459.

14. Brady, H.R. 1994. Adhesion molecules and kidney diseases. *Kidney Int.* 45:1285–1300.

15. McMurray, R.W. 1996. Adhesion molecules in autoimmune disease. *Arthritis Rheum.* 25:215–233.

16. Dustin, M.L., R. Rothlein, A.K. Bhan, C.A. Dinarello, and T.A. Springer. 1986. Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol.* 137:245–254.

17. Dustin, M.L., and T.A. Springer. 1991. Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. *Annu. Rev. Immunol.* 9: 27–66.

18. Bruijn, J.A., E.C. Bergijk, E. de Heer, and C.J. Kootstra. 1994. Molecular adhesion mechanisms and recruitment of inflammatory cells in renal diseases. *Life Sci. Adv.* 13:9–15.

19. Wuthrich, R.P., A.M. Jevnikar, F. Takei, L.H. Glimcher, and V.E. Kelley. 1990. ICAM-1 expression is upregulated in autoimmune lupus nephritis. *Am. J. Pathol.* 136:441–450.

20. Lazaar, A.L., S.M. Albelda, J.M. Pilewski, B. Brennan, E. Pure, and R.A. Panettieri, Jr. 1994. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J. Exp. Med.* 180:807–816.

21. Gonzalo, J.A., C.L. Lloyd, L. Kremer, E. Finger, C. Martinez-A., M.H. Siegelman, M. Cybulsky, and J.C. Gutierrez-Ramos. 1996. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and endothelial adhesion receptors. *J. Clin. Invest.* 98:2332–2345.

22. Horgan, M.J., M. Ge, J. Gu, R. Rothlein, and A.B. Malik. 1991. Role of ICAM-1 in neutrophil-mediated lung vascular injury after occlusion and reperfusion. *Am. J. Physiol. (Heart Circ. Physiol.).* 261:H1578–H1584.

23. Mulligan, M.S., A.A. Vaporciyan, R.L. Warner, M.L. Jones, K.E. Foreman, M. Miyasaka, R.F. Todd III, and P.A. Ward. 1995. Compartmentalized roles for leukocytic adhesion molecules in lung inflammatory injury. *J. Immunol.* 154:1350–1363.

24. Nishikawa, K., Y.J. Guo, M. Miyasaka, T. Tamatani, A.B. Collins, M.-S. Sy, R.T. McCluskey, and G. Andres. 1993. Antibodies to intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 prevent crescent formation in rat autoimmune glomerulonephritis. *J. Exp. Med.* 177:667–677.

25. Xu, H., J.A. Gonzalo, Y. St. Pierre, I.R. Williams, T.S. Kupper, R.S. Cotran, T.A. Springer, and J.-C. Gutierrez-Ramos. 1994. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J. Exp. Med.* 180:95–109.

26. Morgan, A., R.R.C. Buchanan, A.M. Lew, I. Olsen, and N.A. Staines. 1985. Five groups of antigenic determinants on DNA identified by monoclonal antibodies from NZB×NZWF1 and MRL/Mp-lpr/lpr mice. *Immunology.* 55: 75–83.

27. Lloyd, C.M., A.O. Wozencraft, and D.G. Williams. 1993. Cell-mediated pathology in murine malarial nephritis. *Clin. Exp. Immunol.* 94:398–402.

28. Auiti, A., I.J. Webb, C. Bleul, T.A. Springer, and J.C. Gutierrez-Ramos. 1997. The chemokine SDF-1 is a chemoattractant for human CD34$^+$ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34$^+$ cells to peripheral blood. *J. Exp. Med.* 185:111–120.

29. Salant, D.J., and A.V. Cybulsky. 1988. Experimental glomerulonephritis. *Methods Enzymol.* 162:421–461.

30. Soriano, S.G., S.A. Lipton, Y.F. Wang, M. Xiao, T.A. Springer, J.C. Gutierrez-Ramos, and P.R. Hickey. 1996. Intercellular adhesion molecule-1-deficient mice are less susceptible to cerebral ischemia-reperfusion injury. *Ann. Neurol.* 39:618–624.

31. Verdrengh, M., T. Springer, J.C. Gutierrez-Ramos, and A. Tarkowsky. 1996. Role of intercellular adhesion molecule 1 in the pathogenesis of staphylococcal arthritis and in host defence against staphylococcal bacteremia. *Infect. Immun.* 64:2804–2807.

32. Moyer, C.F., J.D. Strandberg, and C.L. Reinisch. 1987. Systemic mononuclear-cell vasculitis in MRL/Mp-lpr/lpr mice. A histologic and immunocytochemical analysis. *Am. J. Pathol.* 127:229–242.

33. Pollak, V.E., and K.S. Kant. 1992. Systemic lupus erythematosus and the kidney. *In* Systemic Lupus Erythematosus. R.G. Lahita, editor. Churchill Livingstone, New York. 683–705.

34. Hunninghake, G.W., and A.S. Fauci. 1979. Pulmonary involvement in the collagen vascular diseases. *Am. Rev. Respir. Dis.* 119:471–503.

35. Wuthrich, R.P. 1992. Vascular adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. *Kidney Int.* 42:903–914.

36. Shlomchik, M.J., M.P. Madaio, N. Donghui, M. Trounstein, and D. Huszar. 1994. The role of B cells in *lpr/lpr*-induced autoimmunity. *J. Exp. Med.* 180:1295–1306.

37. Bernstein, K.A., R.D. Valerio, and J.B. Lefkowith. 1995. Glomerular binding activity in MRL/*lpr* serum consists of antibodies that bind to a DNA/histone/type IV collagen complex. *J. Immunol.* 154:2424–2433.

38. Vlahakos, D.V., M.H. Foster, S. Adams, M. Katz, A.A. Ucci, K.L. Barret, S.K. Datta, and M.P. Madaio. 1992. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int.* 42:1690–1700.

39. Dubois, E.L., and D.L. Tuffanelli. 1964. Clinical manifestations of systemic lupus erythematosus. *JAMA (J. Am. Med. Assoc.).* 19:104–109.

40. Miller, L.R., S.D. Greenberg, and J.W. McLarty. 1985. Lupus lung. *Chest.* 88:265–269.