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# Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane

**(systemic lupus erythematosus/lupus nephritis/autoantibodies/autoimmunity/small nuclear ribonucleoproteins)**

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ABSTRACT **The pathogenesis of systemic lupus erythematosus is thought to be primarily under genetic control, with environmental factors playing a secondary role. However, it has been shown recently that intraperitoneal injection of pristane (2,6,10,14-tetramethylpentadecane) induces autoantibodies typical of lupus in BALB/c mice, a strain not usually considered to be genetically susceptible to the disease. In this study, the induction of autoimmune disease by pristane was investigated. BALB/c mice receiving pristane were tested for autoantibody production and histopathological evidence of glomerulonephritis. Six of 11 mice developed IgM anti-single-stranded DNA antibodies shortly after receiving pristane and 4 developed IgM anti-histone antibodies, but anti-double-stranded DNA antibodies were absent. IgG anti-DNA and anti-histone antibodies were absent. In contrast, the lupus-associated anti-nuclear ribonucleoprotein/Sm and anti-Su autoantibodies produced by these mice were predominantly IgG. In addition to autoantibodies, most of the mice developed significant proteinuria. Light microscopy of the kidney showed segmental or diffuse proliferative glomerulonephritis. Electron microscopy showed subepithelial and mesangial immune-complex deposits and epithelial foot process effacement. Immunofluorescence revealed striking glomerular deposition of IgM, IgG, and C3 with a mesangial or mesangiocapillary distribution. Thus, pristane induces immune-complex glomerulonephritis in association with autoantibodies typical of lupus in BALB/c mice. These data support the idea that lupus is produced by an interplay of genetic and environmental factors and that unlike the MRL or (NZB × W)$F_1$ mouse models, in which genetic susceptibility factors are of primary importance, environmental factors are of considerable importance in the autoimmune disease of pristane-treated BALB/c mice.**

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by anti-nuclear antibodies, immune-complex glomerulonephritis, arthritis, and other manifestations. Anti-double-stranded (ds) DNA autoantibodies are highly specific for SLE and may play a key role in the pathogenesis of immune-complex nephritis in lupus (1, 2). However, autoantibodies to glomerular antigens (3) and/or dysregulated cytokine production (4, 5) may also be involved. Human SLE is influenced strongly by major histocompatibility complex-linked and -nonlinked genes (6–8). Multiple genetic loci that accelerate the onset of autoantibody production and/or nephritis also have been identified in murine lupus models (9, 10). The importance of environmental factors in the pathogenesis of lupus is less clear. However, the role of environmental exposures in autoantibody production is underscored by the recent demonstration that intraperitoneal (i.p.) injection of pristane (2,6,10,14-tetramethylpentadecane) induces autoantibodies characteristic of SLE, including anti-Su and anti-nuclear ribonucleoprotein (nRNP)/Sm, in BALB/c mice, a strain not usually considered to be predisposed to autoimmunity (11). Titers of these autoantibodies are comparable to those found in MRL/*lpr* mice (12). The present data show that in addition to IgG anti-Su and anti-nRNP/Sm autoantibodies, pristane induces IgM anti-single-stranded (ss) DNA, anti-histone antibodies, and immune-complex glomerulonephritis in the "nonautoimmune" BALB/c strain.

## MATERIALS AND METHODS

**Administration of Pristane.** Eleven 4- to 5-month-old and 10 2.5-month-old female BALB/c ByJ mice (The Jackson Laboratory) received a single i.p. injection of 0.5 ml of pristane (Sigma) (11). Sera were obtained at 1, 2, and 4 weeks and monthly thereafter. Urine samples were tested monthly for protein concentration by using Albustix reagent strips (Miles).

**ELISAs for Anti-nRNP/Sm, Su, ssDNA, and Histone Autoantibodies.** Anti-Su and anti-nRNP/Sm antigen-capture ELISAs were performed as described (12) with 1:250 diluted murine serum and alkaline phosphatase-conjugated goat anti-mouse IgG or IgM antibodies. Antibodies to heat-denatured calf thymus DNA (ssDNA, from Sigma) and to total calf thymus histones (United States Biochemical) were detected by ELISAs as described (13, 14) with a 1:500 dilution of murine sera and alkaline phosphatase-conjugated goat anti-mouse IgG or IgM antibodies.

**Light and Electron Microscopy.** Six months after receiving pristane, BALB/c and control mice not receiving pristane were anesthetized and fixed by perfusion through the left ventricle (15). The inferior vena cava was nicked below the renal veins, and 20 ml of saline was perfused slowly, followed by 10 ml of 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4/4 mM $CaCl_2$. For light microscopy, 3-$\mu$m sections of aldehyde-fixed renal cortex were stained with hematoxylin and eosin as described (16). For electron microscopy, aldehyde-fixed renal tissue was postfixed with osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812. Thin sections (60 nm) were stained with lead citrate and uranyl acetate and examined by electron microscopy (16).

**Immunofluorescence.** Kidneys were excised from pristane-primed or control mice and snap-frozen in isopentane chilled in liquid $N_2$. Cryostat sections (4 $\mu$m) were stained with a 1:40 dilution of fluorescein isothiocyanate (FITC) or rhodamine-conjugated goat anti-mouse IgM, IgG, IgG1, IgG2a, IgG2b, or IgG3 antibodies (Southern Biotechnology Associates) or with FITC-conjugated rabbit anti-mouse C3 antiserum (Organon

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Abbreviations: nRNP, nuclear ribonucleoprotein; SLE, systemic lupus erythematosus; ds, double stranded; ss, single stranded; IL, interleukin.

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Teknika–Cappel) at the same dilution. Stained sections were examined with an epifluorescence UV microscope with FITC and rhodamine filters.

## RESULTS

SLE is associated with autoantibodies to protein or protein–nucleic acid complexes (17), including the U1 small nRNP (anti-nRNP and Sm antibodies) (18), Su antigen (19, 20), and nucleosomes (anti-DNA and anti-histone antibodies) (21). We have shown (11) that anti-Su and anti-nRNP/Sm antibody responses develop in BALB/c mice, a "nonautoimmune" strain, within several months after a single i.p. pristane injection (11). The present study addresses the question of whether autoantibody production induced by pristane is associated with autoimmune disease compatible with SLE.

**IgG Anti-Su and Anti-nRNP/Sm Autoantibodies.** IgG anti-Su antibodies appeared in the sera of 5 of 11 BALB/c mice 2–3 months after pristane injection (Fig. 1*D*). IgM anti-Su antibodies were detected at a low level in only 1 of 5 mice (Fig. 1*B*), and there was little evidence for IgM to IgG class switching. Six of the mice never developed anti-Su antibodies. IgM and IgG anti-nRNP/Sm antibodies exhibited a similar pattern (Fig. 1 *A* and *C*), although their onset was delayed compared with anti-Su antibodies, as reported (11). As with anti-Su, IgG antibodies dominated the immune response to nRNP/Sm, with little evidence for class switching. The unexplained lack of autoantibody class switching in lupus has been noted (22). Seven of 11 mice developed anti-nRNP/Sm by 6 months after pristane injection, and there was little or no correlation between anti-Su and anti-nRNP/Sm antibody production by individual mice. Analysis of the isotypes of anti-Su and anti-nRNP/Sm antibodies by ELISA showed that IgG1, IgG2a, IgG2b, and IgG3 autoantibodies of both specificities were present (data not shown).

**IgM Anti-DNA and Anti-Histone Antibodies.** Anti-ssDNA antibody production contrasted markedly with that of anti-Su and nRNP/Sm. IgM anti-ssDNA antibodies appeared in 4 of 11 BALB/c mice within 1 month of pristane injection (Fig. 2*A*), and in two more mice by 3 months. In a second experiment, they were found in 4 of 10 sera as early as 1–2 weeks after administering pristane (data not shown). In contrast to the anti-Su/nRNP/Sm antibodies, IgG anti-ssDNA antibodies were undetectable (Fig. 2*C*). There was no apparent relationship between IgM anti-ssDNA and IgM or IgG anti-Su or nRNP/Sm autoantibody production (cf. Figs. 1 and 2). Anti-dsDNA antibodies were absent in all sera by the *Crithidia luciliae* kinetoplast staining method.

The production of IgM anti-histone antibodies resembled anti-ssDNA autoantibody production in its early onset and the absence of IgG class antibodies (Fig. 2 *B* and *D*). There was no association of anti-histone with anti-Su or anti-nRNP/Sm antibodies. Unexpectedly, there was also no relationship between anti-ssDNA and anti-histone antibody production (in 6 of 11 and 3 of 11 mice, respectively), despite previous evidence that nucleosomes (DNA plus histones) are a major target of immune responses in murine lupus (21). Thus, the data suggest that BALB/c mice produce the lupus-associated autoantibodies anti-Su and anti-nRNP/Sm, as well as less disease-specific autoantibodies such as anti-ssDNA and anti-histone. Whereas the anti-Su/nRNP/Sm antibody responses were predominantly IgG, the anti-ssDNA and anti-histone responses were limited to IgM. In view of the association of autoantibody production with nephritis in SLE, kidneys of the mice were examined for immune-complex glomerulonephritis.

**Development of Immune-Complex Glomerulonephritis.** Light microscopy of kidney sections from five mice injected with pristane 6 months earlier revealed segmental or diffuse proliferative glomerular lesions (Fig. 3*A*), consistent with World Health Organization class III, IV, and V lupus nephritis (23). Proliferation was mainly related to an increase in mesangial cells. In addition, the epithelial and endothelial cells were hypertrophic and capillary loops were thickened. A mild to moderate influx of monocytes was seen in the glomerular capillaries. These changes were not apparent in the glomeruli of control mice (cf. Fig. 3 *A* vs. *C*), which exhibited the usual glomerular morphology (24). Electron microscopy showed irregular thickening of the glomerular basement membrane, subepithelial dense deposits, and epithelial foot process effacement in pristane-treated mice (Fig. 3*B*) but not in control mice (Fig. 3*D*). The mesangial matrix and mesangial cells were increased, and scattered mesangial deposits were also noted. Immunofluorescence studies reinforced the morphologic findings (Fig. 4). The glomeruli from pristane-primed mice re-

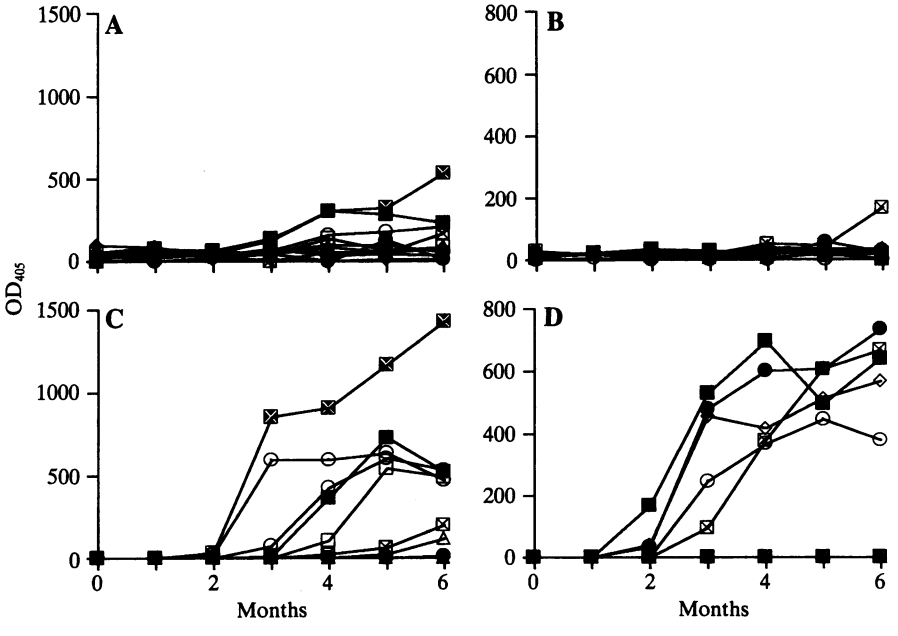


FIG. 1. Time course of anti-nRNP/Sm and Su antibody production. Levels of IgM (*A* and *B*) and IgG (*C* and *D*) anti-nRNP/Sm (*A* and *C*) and anti-Su (*B* and *D*) antibodies in sera from 11 BALB/c mice injected i.p. with pristane were determined by antigen-capture ELISA. $OD_{405} \times 10^{-3}$ is plotted as a function of time (months) after pristane was injected.

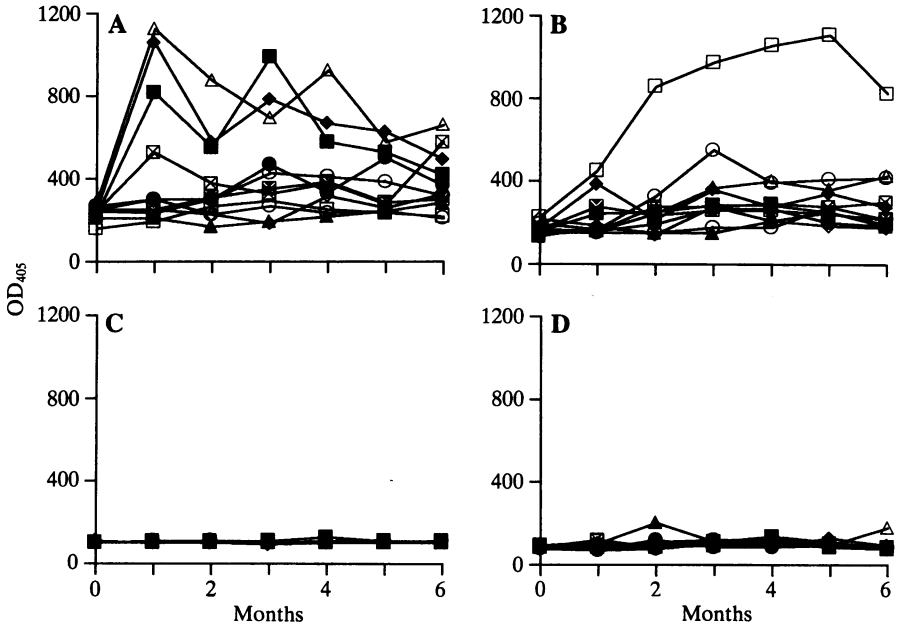


FIG. 2. Time course of anti-ssDNA and anti-histone antibody production. Levels of IgM (*A* and *B*) and IgG (*C* and *D*) anti-ssDNA (*A* and *C*) and anti-histone (*B* and *D*) antibodies in sera from 11 BALB/c mice injected i.p. with pristane were determined by ELISA. $OD_{405} \times 10^{-3}$ is plotted as a function of time (months) after pristane was injected.

vealed mesangial or mesangiocapillary reactivities for IgG, IgM, and C3 (Fig. 4 *H*, *M*, and *N*, respectively), whereas glomeruli from control mice were negative for IgG and showed very weak reactivity for IgM and C3 (Fig. 4 *A*, *F*, and *G*).

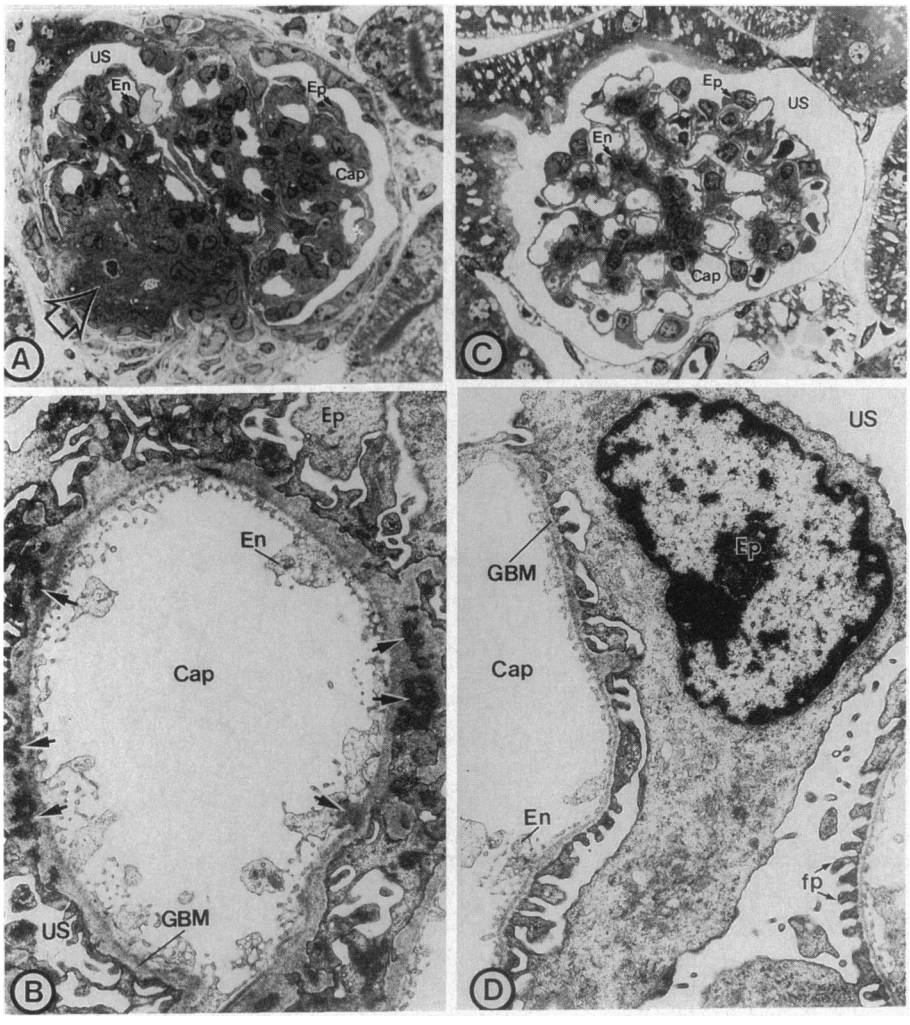


FIG. 3. Morphology of glomerulonephritis. Light micrographs of a renal glomerulus from a BALB/c mouse injected 6 months previously with pristane (*A*) and from a control mouse (*C*). Arrow in *A* indicates segmental proliferative lesions with effacement of capillaries and cellular elements. In other regions, mesangial proliferation and capillary wall thickening are evident. US, urinary space; En, endothelium; Ep, epithelium; Cap, capillary lumen. (×375.) Electron microscopy of renal glomerular capillary wall from a BALB/c mouse injected 6 months previously with pristane (*B*) and from a control mouse (*D*). Arrows in *B* indicate subepithelial electron-dense immune-complex deposits in the irregularly thickened glomerular basement membrane (GBM). Note effacement of epithelial foot processes (fp) in glomeruli from pristane-primed mouse (*B*). (×7500.)

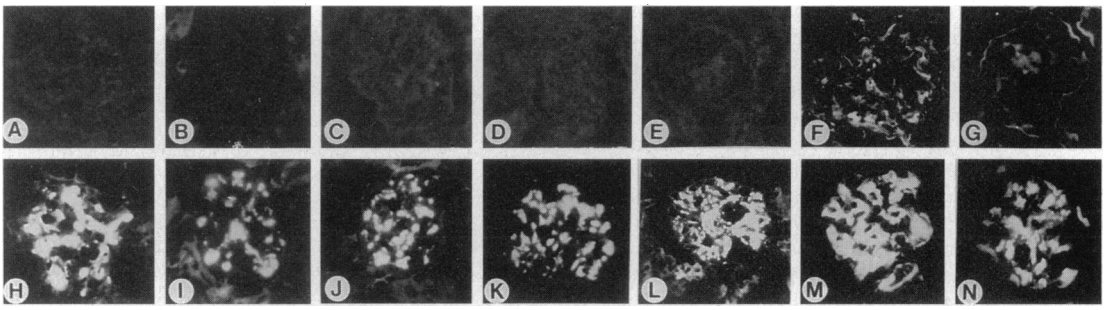


FIG. 4. Immunofluorescence microscopy. Photomicrographs of glomeruli from a control mouse (*A–G*) or a BALB/c mouse injected 6 months previously with pristane (*H–N*), stained with specific goat anti-mouse immunoglobulin or complement antibodies. Note the predominant mesangial or mesangiocapillary distribution of the immune complex deposits in the pristane-induced glomerular lesions. (*A* and *H*) Anti-IgG. (*B* and *I*) Anti-IgG1. (*C* and *J*) Anti-IgG2a. (*D* and *K*) Anti-IgG2b. (*E* and *L*) Anti-IgG3. (*F* and *M*) Anti-IgM. (*G* and *N*) Anti-C3. (×120.)

Staining with isotype-specific second antibodies revealed glomerular immunoreactivity with IgG1, IgG2a, IgG2b, and IgG3 in the pristane-primed (Fig. 4 *I–L*, respectively) but not control (Fig. 4 *B–E*) mice. Urinalysis revealed 1-3+ proteinuria (30–300 mg/dl) in all mice at 6 months after pristane injection. Proteinuria was ≤1+ (30 mg/dl) in control mice.

## DISCUSSION

Autoantibodies associated with SLE are produced by BALB/c mice treated with pristane (11, 12) and by pristane-primed (NZB × BALB/c)$F_1$ mice injected with NZB myeloma cells (25). In addition, ≈10% of paraproteins produced by pristane-induced mouse myelomas are reactive with nucleic acids and/or dinitrophenyl derivatives (26), and pristane induces rheumatoid factor and autoantibodies to type II collagen and hsp70 (27, 28). The present studies indicate that pristane also causes renal changes in BALB/c mice compatible with lupus nephritis.

**Induction of Anti-DNA and Anti-Histone Antibodies.** In contrast to IgG anti-Su/nRNP/Sm autoantibodies, which appear 2–6 months after pristane injection (Fig. 1 and refs. 11 and 12), anti-ssDNA and anti-histone antibodies appeared as early as 1–4 weeks (Fig. 2) and were exclusively IgM class. The absence of IgG anti-ssDNA or anti-histone antibodies may imply that these specificities develop by a different mechanism than was responsible for the IgG anti-Su/nRNP/Sm antibodies. Low-affinity polyreactive anti-ssDNA antibodies, primarily IgM class, are produced by the B1 (CD5+) B-cell subset, whereas high-affinity IgG autoantibodies are produced mainly by the CD5− subset (29–31). Of note, the B1 subset is highly enriched in the peritoneal cavity and is expanded in BALB/c and NZB mice (32), raising the possibility that the IgM anti-ssDNA and anti-histone antibodies induced by i.p. pristane injection were derived from that subset. Moreover, preliminary competitive binding experiments suggest that the IgM anti-ssDNA antibodies have low affinity (data not shown). In contrast, the anti-Su/nRNP/Sm autoantibodies induced by pristane were predominantly of the T-cell-dependent subclasses IgG1, IgG2a, or IgG2b (11, 12). In view of the suggestion that antibody production by the CD5+ subset may be largely T-cell independent (33), the anti-Su/nRNP/Sm antibodies may have been derived from CD5− B cells. However, further studies are needed to answer this question definitively.

**Pathogenesis of Glomerulonephritis in Pristane-Treated Mice.** There is considerable evidence that anti-DNA antibodies are involved in the pathogenesis of glomerulonephritis (1, 2, 34). However, although some anti-ssDNA antibodies may be pathogenic (34, 35), the bulk of evidence suggests that anti-dsDNA antibodies are more relevant to the pathogenesis of lupus nephritis (36). Thus, the absence of anti-dsDNA antibodies in the sera of pristane-treated BALB/c mice suggests that the severe immune complex nephritis in these mice was not mediated by anti-DNA antibodies. This interpretation is supported further by the fact that some anti-DNA-antibody-negative mice also developed glomerulonephritis. However, we cannot exclude the possibility that polyclonal activation of CD5+ B cells is essential for the later development of high-affinity IgG autoantibodies and lupus-like disease (37) in pristane-treated mice, even though the IgM anti-ssDNA antibodies produced by these cells are not pathogenic. It is of interest, in this regard, that CD5+ B cells are an important source of interleukin (IL) 10 (38), a cytokine that has been implicated in the pathogenesis of glomerulonephritis (39).

In addition to promoting plasmacytoma growth in mice treated with pristane (40–42), IL-6 may play a role in mesangial proliferative and membranoproliferative glomerulonephritis (43, 44), and murine lupus nephritis (4, 5). Since the nephritis in pristane-primed BALB/c mice was similar histologically to that in the (NZB × W)$F_1$ model, it is possible that increased IL-6 levels exacerbated glomerulonephritis in mice receiving pristane. IL-10 also exacerbates glomerulonephritis in (NZB × W)$F_1$ mice (39). It will be of interest to investigate whether increased IL-6 or IL-10 production, the latter possibly due to the CD5+ B-cell expansion in BALB/c mice (38), is involved in the striking mesangial cell proliferation seen in both the pristane and (NZB × W)$F_1$ models.

**Relevance to Idiopathic SLE.** These studies provide evidence that the autoimmune syndrome induced by pristane in BALB/c mice resembles idiopathic SLE in humans. As in human SLE, mice injected with pristane develop anti-nuclear antibodies recognizing a small subset of antigens including the Su antigen, components of U small nRNPs, ssDNA, and histones. The titers of anti-Su and anti-nRNP/Sm autoantibodies produced by pristane-primed BALB/c mice are at least as high as seen in MRL/*lpr* mice (12). However, unlike human SLE, dsDNA, Ro (SS-A), and La (SS-B) are not prominent autoantigens in this model.

The development of glomerular lesions reminiscent of lupus nephritis (Figs. 3 and 4) and autoantibodies characteristic of SLE suggests that environmental stimuli may precipitate autoimmune disease in mice not genetically predisposed to SLE. That idea is also supported by the induction of autoantibodies, and in some cases glomerular lesions, by $HgCl_2$ or lipopolysaccharide (45–48). The relatively high concentration of pristane in certain food items (49) raises the possibility that environmental exposure to pristane or its metabolites might contribute to the pathogenesis of some cases of human SLE. Ingestion of pristane causes amyloidosis in mice (50), but it is not known whether autoimmunity develops after oral exposure. It should be noted that the renal lesions in mice receiving pristane i.p. were not compatible with amyloidosis.

Multiple genes contribute to SLE susceptibility (8, 9), and environmental factors are generally relegated to a secondary role. If, as suggested by our data, pristane treatment bypasses

or replaces the effect of one or more genetic susceptibility factors, then understanding the pathogenesis of pristane-induced lupus-like syndrome may simplify the identification of genes conferring susceptibility to idiopathic lupus. Furthermore, the induction by pristane of a lupus-like disease in other "normal" strains of mice in addition to BALB/c (data not shown) suggests that the notion that SLE is primarily a genetic disease may be overly simplistic. Our data suggest that in some instances, such as the MRL or (NZB × W)$F_1$ mouse models, genetic susceptibility factors are primarily responsible for SLE (9), whereas in other cases, such as the pristane model, environmental factors may be of considerable importance. The two situations may be analogous to familial and sporadic lupus, respectively. Intermediates between the two extreme cases may exist. It will be of interest in the future to examine whether the genetic and environmental factors influence susceptibility to SLE independently or through actions on a common pathway.

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