LACK OF RELATIONSHIP BETWEEN SERUM gp70 LEVELS AND THE SEVERITY OF SYSTEMIC LUPUS ERYTHEMATOSUS IN MRL/l MICE

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Murine lupus is characterized by multiple autoimmune responses in each of the several kinds of mice in which it occurs (MRL/l, BXSB and NZB × W) (1). Because of this multiplicity of autoimmune responses, it is difficult to determine the pathogenetic importance of any one individually. Among the numerous responses observed, two seem most closely related to the course of the disease in all three kinds of mice; anti-DNA and anti-gp70 (2).

gp70 is a serum protein found in all mice. It is a 70,000 mol wt glycoprotein immunologically closely related to the surface glycoprotein of NZB-type endogenous murine retrovirus (3). However, it is not produced as part of a retroviral expression, but rather is formed primarily in the liver, and behaves as an acute-phase protein (4). It is present in moderate to high (30–100 μg/ml) levels in all lupus mice and several immunologically normal strains, and at lower levels (0.5–5 μg/ml) in most normal strains (5). All lupus mice, and only lupus mice, appear to make an autoimmune response to gp70 that results in circulating gp70–anti-gp70 immune complexes, and immune deposits in their glomeruli and blood vessels apparently participate in the immunologically-induced glomerulonephritis and vasculitis (5).

In an attempt to determine the importance and/or essentiality of the anti-gp70 autoimmune response in murine lupus, we undertook the development of low gp70 lines of MRL/l mice. This was accomplished by crossing MRL/l with BALB/c, a very low-gp70 line, and backcrossing the F_1 hybrids to MRL/l mice for 11 generations, selecting for low serum gp70 at each generation. After 11 backcrosses, brother × sister matings were used to establish low gp70–MRL/l congenic lines.

Materials and Methods

Mice. MRL/l mice from the Scripps Clinic breeding colony were crossed with BALB/c mice from The Jackson Laboratories (Bar Harbor, ME). The resultant F_1 hybrids were backcrossed to MRL/l mice for 11 generations, with intercrossing at several of

This is publication 4175IMM from the Department of Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037. The work reported herein was supported, in part, by grants AI07007, AG01743, CA27489, AM33826, and AM31203 from the National Institutes of Health, Bethesda, MD.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/86/2/0459/05 $1.00
Volume 163 February 1986 458–462

the later generations, and continuously after N11. The offspring of each mating were bled at ~1 mo of age, and those with the lowest levels of serum gp70 were selected as breeders. Breeding has continued past N11 F_7, and two lines, L7 and L8, are currently being maintained. Female mice from all three groups (MRL/l, MRL/l-low gp70, and BALB/c) were selected for use in this study.

Serology. Mice were bled at 2, 3, and 4 mo of age, and at autopsy from the retroorbital sinus. Serum gp70 levels were determined by RIA as described previously (5). Levels of gp70-anti-gp70 immune complexes were determined by absorbing diluted serum samples with staphylococcal protein A (Calbiochem-Behring, La Jolla, CA), and the change in the amount of gp70 detected by RIA before and after absorption was calculated. Serum levels of anti-single-stranded DNA (ssDNA) were determined by a modification of the Farr DNA-binding immunoassay as described previously (6). Total concentrations of serum IgG levels were measured using radial immunodiffusion as previously described (7). A modification of the Raji cell assay previously described (8) was used to test for total immune complexes in sera.

LPS Stimulation. Female MRL/l and MRL/l-low gp70 mice were injected with 50 μg of LPS (*Salmonella minnesota* Re595) in 0.4 ml saline i.p. They were bled three times from the retroorbital sinus 3 h preinjection, and 24 and 72 h postinjection. The sera were analyzed for gp70.

Immunofluorescence Studies. Kidney sections from MRL/l-low gp70 mice and parental strains were tested for the presence of IgG, C3, and gp70 deposits using the methods outlined previously (3, 9). FITC-conjugated $F(ab')_2$ goat anti-mouse IgG or FITC-conjugated $F(ab')_2$ goat anti-mouse C3 was bound directly to kidney tissue to test for IgG or C3 deposition. In testing for gp70 deposits, tissue was incubated first with goat anti-Rauscher gp70, and then with FITC-conjugated rabbit anti-goat IgG.

H2 Antisera. Anti-$H2^k$ and anti-$H2^d$ antisera produced in H2-incompatible F_1 hybrids had a nonspecific binding of <5% and a specific binding of >50% at a 1:200 dilution of each antiserum.

H2 Assay. Mice were bled from the retroorbital sinus using heparinized capillary tubes. The blood was layered over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ), and centrifuged to extract the red blood cells used in the assay. Cells were incubated with both antisera at a 1:200 dilution, washed, and incubated with 5 ng ^{125}I-staphylococcal protein A. Cells were washed again, and the final pellets were counted.

Histology. MRL/l and MRL/l-low gp70 mice were sacrificed when moribund, or at 5.5 mo of age. BALB/c mice were chosen at 3-5 mo of age for age-related normal controls. For histologic purposes, cervical and mesenteric lymph nodes, ovaries, spleens, kidneys, livers, hearts, thymuses, and lungs were taken, as were hind and forelegs from MRL/l and MRL/l-low gp70 mice. Lymph nodes, heart, and spleen were weighed. Blood urea nitrogen (BUN) levels at autopsy were determined using AZOSTIX reagent strips (Ames Division, Miles Laboratories, Inc., Elkhart, IN). Tissues and organs of interest were fixed in Bouin's solution for 48 h, then held in 70% ethanol until processing. Bones were decalcified in decalcifying solution (American Scientific Products, McGaw Park, IL) for 2 d after fixing. Soft tissues were stained with periodic acid-Schiff, and bones with H & E.

Statistical Analysis. Data were analyzed by the Student's *t*-test.

Results

By repeated backcrossing of (MRL/l × BALB/c)F_1 hybrids to MRL/l mice, we have developed several MRL/l-low gp70 congenic lines, the genomes of which should be >99.97% MRL/l. Levels of serum gp70 in the MRL/l-low gp70 congenic females (3.0 μg/ml, Table I) are significantly lower ($p < 0.001$) than those of MRL/l females of approximately the same age (29.6 μg/ml), though not as low as the BALB/c (0.7 μg/ml) from which the cross was derived. The amount of gp70 removed from sera by staphylococcal protein A absorption (an indirect

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TABLE I
Serological Parameters in MRL/l-low gp70 and Parental Strains of Mice

Strain*	gp70	gp70 IC	Raji IC	IgG	Anti-ssDNA binding
	μg/ml	*μg/ml*	*μg AMG/ml*	*mg/ml*	%
BALB/c	0.7 ± 0.1	0.1 ± 0	0.8 ± 0	2.0 ± 0.1	17 ± 1
MRL/l	29.6 ± 3.0	8.9 ± 2.9	152 ± 15	13.8 ± 1.0	53 ± 3
MRL/l-low gp70	3.0 ± 0.2	0.5 ± 0.2	146 ± 15	14.7 ± 0.8	72 ± 3

Data are expressed as mean ± SE.
* Age, 2–4 mo; *n*, 19–40.

measure of gp70–anti-gp70 immune complexes) is also much lower in the congenic strain.

Both MRL/l and MRL/l–low gp70 mice show response to LPS stimulation. MRL/l females increased from 44.7 to 109.8 μg/ml in 24 h postinjection, while the MRL/l–low gp70 females showed a quantitatively lower but proportionally similar response, increasing from 2.5 to 4.2 μg/ml in the same period.

A comparison of levels of IgG, anti-ssDNA, and immune complex (Raji) (Table I) indicates that the BALB/c strain is significantly different from both MRL/l and MRL/l–low gp70 mice in all three factors ($p < 0.001$). The MRL/l–low gp70 is not different ($p > 0.5$) from the MRL/l strain except in anti-ssDNA ($p < 0.001$), where there is a higher percent binding in the new congenic than in the parental strain.

Amounts of IgG and C3 deposited in glomeruli of MRL/l and MRL/l–low gp70 mice are the same (Fig. 1), while BALB/c females show no detectable deposits. gp70 deposits evident in the MRL/l were lacking in both the MRL/l–low gp70 and BALB/c mice (Fig. 1).

All MRL/l–low gp70 mice tested were $H2^k$. The parental strains, BALB/c ByJ and MRL/l are $H2^d$ and $H2^k$, respectively. 50% mortality occurred at approximately the same time in the MRL/l–low gp70 mice as the MRL/l (149 and 153 d), and considerably earlier in both than in the BALB/c ByJ females (>672 d).

At autopsy, BUN, degree of glomerulonephritis, and incidence of arthritis and myocardial infarct were the same in both the MRL/l–low gp70 and MRL/l mice, and differed from BALB/c ByJ (Table II). Mesenteric lymph node, heart, and whole body weights were comparable in both MRL/l congenics, and differed from the BALB/c ByJ ($p < 0.001$), but spleen and cervical lymph node weights in the MRL/l–low gp70 strain were intermediate between the other two groups (Table II).

Discussion

In the study of murine SLE, various serological factors show a good level of correlation among themselves and with survival (2). In this study, we attempted to remove one of these serological factors, gp70 immune complexes, and leave the rest intact. This has been possible with the MRL/l strain.

Serum levels of gp70 in the MRL/l–low gp70 congenic are low, and remain low even with stimulation by LPS. Serum levels of total IgG and immune complex (Raji) remain unchanged, and levels of anti-ssDNA are, if anything, slightly

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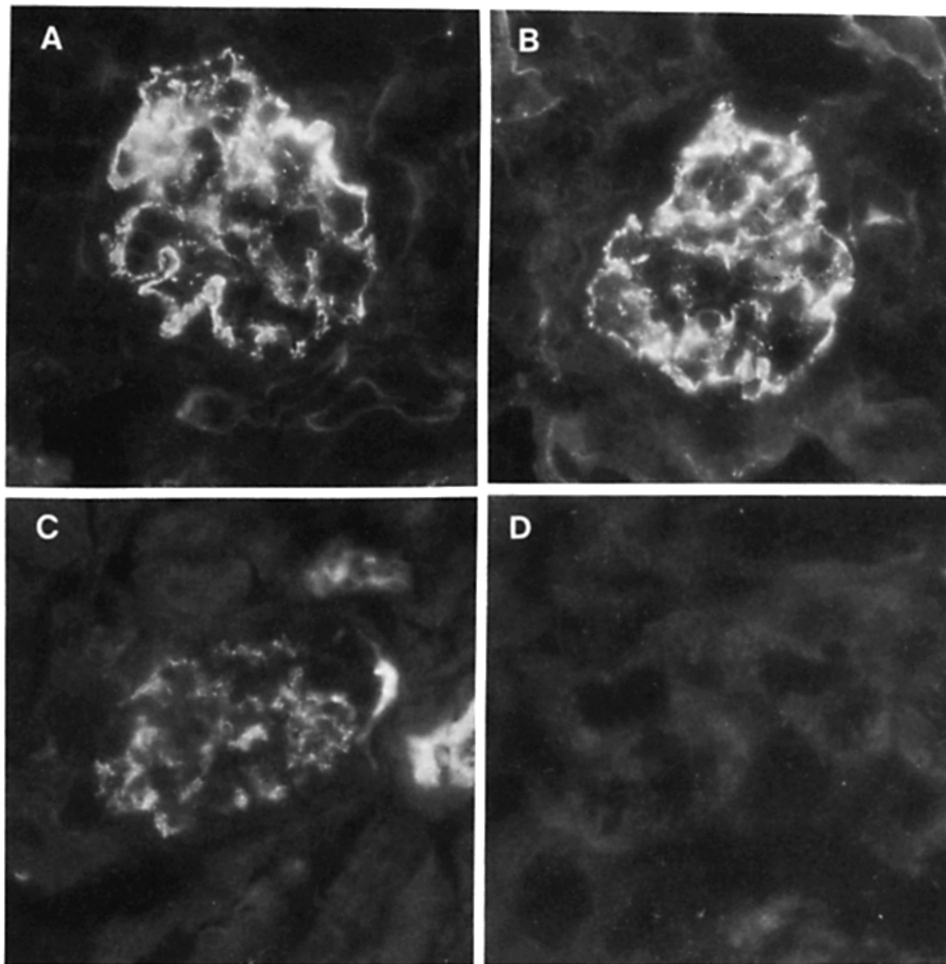

FIGURE 1. Immunofluorescence patterns in kidney glomeruli of high and low gp70–MRL/l mice. (*A*) High gp70 animal, anti-IgG stain. (*B*) Low gp70 animal, anti-IgG stain. (*C*) High gp70 animal, anti-gp70 stain. (*D*) Low gp70 mouse, anti-gp70 stain.

TABLE II

Pathological Features in MRL/l–low gp70 and Parental Strains Aged 3–6 mo

Mouse strain	Number of animals used (*n*)	BUN (mg/dl)	Glomerulonephritis*	Myocardial infarction (%)	Histological evidence of arthritis (%)	Organ weight (g)				
						Spleen	Mesenteric lymph node	Cervical lymph node	Heart	Body
BALB/c	11	10 ± 0	1 ± 0	0	—‡	115 ± 6	39.6 ± 4	3.8 ± 0	126 ± 6	20.7 ± 1
MRL/l	11	38 ± 6	3 ± 0.3	18	27	624 ± 89	1,035 ± 164	371 ± 46	179 ± 6	32.9 ± 1.5
MRL/l-low gp70	12	33 ± 7	3 ± 0.3	17	25	347 ± 43	798 ± 97	187 ± 24	185 ± 7	30.8 ± 1.4

* Severity scale of 1–4.

‡ Since BALB/c mice have no history of arthritis, no joints were examined histologically.

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higher than in the MRL/l strain. Deposition of IgG and C3 in renal glomeruli, presumably as part of immune complexes, is similar to that found in MRL/l mice, but gp70 deposits are absent. This indicates that not only is gp70 greatly reduced in the bloodstream, but also it has not been detectably bound in the glomeruli.

The low gp70 congenics resembled the MRL/l mice from which they were derived in almost all respects. Course and character of disease and autopsy findings in both were indistinguishable except for somewhat smaller spleen and cervical lymph nodes in the low gp70–MRL/l mice.

Summary

In the MRL/l mouse, gp70 apparently plays a role as an autoantigen in the development of SLE. However, while gp70 may be an important pathogenetic element, it is not essential to MRL SLE, since elimination of most of the serum gp70 and virtually all of the immune complex gp70 from MRL/l–low gp70 congenic lines had no observable effect on the course or nature of the disease. Thus, while gp70 in the MRL/l mouse appears to be a convenient autoantigenic target when present in significant levels, in its absence the host appears capable of directing its aberrant immunologic responsiveness elsewhere with undiminished pathogenicity.

Received for publication 7 November 1985.

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