Brief Definitive Report

INDUCTION OF SEVERE AUTOIMMUNE DISEASE IN NORMAL MICE BY SIMULTANEOUS ACTION OF MULTIPLE IMMUNOSTIMULATORS

BY LEMING HANG, M. TERESA AGUADO, FRANK J. DIXON, AND ARGYRIOS N. THEOFILOPOULOS

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Mice with late-life systemic lupus erythematosus (SLE) backgrounds develop early, severe disease when subjected to various accelerating factors. Similar immunostimulation of normal mice leads to relatively innocuous autoimmune responses (1–4).

This study asks the following questions: (*a*) Can the resistance of immunologically normal mice to immunostimulation and pathogenic autoimmune responses be overcome by simultaneous action of multiple qualitatively different immunostimulating factors, and (*b*) if so, what features distinguish the pathogenic vs. nonpathogenic autoimmune responses? We found that any single accelerating factor can enhance the underlying autoimmune diseases in genetically predisposed mice, but simultaneous stimulation by multiple accelerating factors is required to induce pathogenic autoimmune responses in immunologically normal mice. The severity of pathogenic autoimmune responses correlates best with the total quantities of circulating immune complexes (CIC) and the fine characteristics of the autoantibodies rather than the magnitudes or multitudes of autoimmune responses elicited.

Materials and Methods

MRL-*lpr/lpr* (lymphoproliferation gene homozygotes; MRL-*lpr*), C57BL/6-*lpr/lpr* (B6-*lpr*), and C3H/HeJ-*lpr/lpr* (C3H-*lpr*) female mice were originally developed at The Jackson Laboratory (Bar Harbor, ME) (1) and bred further at the Scripps Mouse Colony. The non-*lpr* congenic MRL/n, B6, and C3H/HeJ mice were bred and maintained at the Scripps Mouse Colony. Groups of 15 female mice of the three *lpr* substrains and their non-*lpr* congenic strains were studied.

Five groups of mice (two *lpr* strains, B6-*lpr* and C3H-*lpr*, and three non-*lpr* strains, MRL/n, B6, C3H/HeJ) were injected intraperitoneally with the lipid-A portion of lipopolysaccharide (LPS) (R595, purified from *Salmonella minnesota*; Calbiochem-Behring Corp., San Diego, CA) from 6 wk of age until moribund, or up to 10 mo, as previously described (3). Similar groups of unmanipulated mice served as controls. Histologic and serologic studies in the above groups of mice were performed as previously described (5, 6).

This is publication number 3438IMM from the Department of Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA, 92037. This work was supported in part by grants AI-07007, AM-31023, and AM33826, from the National Institutes of Health, and by the Kroc Foundation. L. Hang is recipient of a Lupus Research Institute Scholarship. M. T. Aguado is recipient of an Arthritis Foundation Postdoctoral Fellowship.

TABLE I

Effects of Immunostimulation on Survival and Autoimmune Histopathologic Manifestations in Three Different Murine Strains

Murine strain background	Mode of immunostimulation	Age of 50% mortality	Lymphoproliferation		Histopathology	
			Lymphoid mass	Spleen mass	GN	Arthritis
		mo	*g*	*g*		
MRL/n	*lpr*	5.5	1.9	0.69	3–4+	2–3$^+$
	LPS	6.5	<0.1	0.45	3–4+	0
	None	17	<0.1	0.38	3–4+	1$^+$
C57BL/6	*lpr* + LPS	7.5	1.2	0.58	3–4+	0
	lpr	12	0.8	0.33	1–2+	0
	LPS	>12	<0.1	0.38	1–2+	0
	None	>17	<0.1	0.12	0	0
C3H/HeJ	*lpr* + LPS	>12	1.8	0.60	0–1+	0
	lpr	>12	1.7	0.58	0–1+	0
	LPS	>12	0.1	0.15	0	0
	None	>17	0.1	0.14	0	0

In each group, autopsies were done on mice that appeared moribund (not necessarily due to GN), or at 12 or 17 mo of age. The degree of lymphoproliferation and severity of histopathology in each group represent average values obtained with 15 mice.

Results

As indicated in Table I, immunostimulation by *lpr* accelerated the latent disease (glomerulonephritis [GN] and arthritis) of SLE-prone MRL/n mice (50% mortality reduced from 17 to 5.5 mo). In contrast, *lpr* had much less disease-inducing effect on C567BL/6 and C3H/HeJ mice. Even at 12 mo, these mice had only mild GN and no arthritis, and did not die from kidney failure.

Chronic immunostimulation by LPS accelerated the latent autoimmunity and GN (though not the arthritis) of MRL/n mice (50% mortality at 6.5 mo), but induced much milder SLE in LPS-responding normal mice (B6), and none in LPS-nonresponding normal mice (C3H/HeJ). When subjected to simultaneous *lpr* and LPS immunostimulation, normal mice (B6) developed early-onset, fatal SLE (50% GN-associated mortality at 7.5 mo).

The total lymphoid mass (at 5 mo), reflecting the degree of *lpr*-induced lymphoid hyperplasia, was highest in MRL-*lpr*, followed closely by C3H-*lpr* mice. B6-*lpr* mice had less lymphoproliferation, although proliferation increases to comparable levels with age or simultaneous LPS treatment. LPS-induced lymphoproliferation was manifested by weight increases in the spleen, rather than in lymph nodes.

Each of *lpr* and LPS increased the total IgG in all three strains, except in the case of C3H/HeJ injected with LPS (Table II). In SLE-prone MRL/n mice, *lpr* induced the highest increases of IgG, particularly IgG1 and IgG2a, while LPS induced smaller increases, predominantly in IgG2b. In the two normal strains, *lpr* induced a smaller IgG increase, but the increase was in the same IgG subclasses as in MRL. In the LPS responder, B6, the LPS-associated increase was predominantly in the IgG2b subclass. With superimposed LPS stimulation, B6-*lpr* mice attained IgG levels nearly equivalent to MRL-*lpr* mice, but the predominant IgG isotype was IgG2b.

In MRL mice, *lpr* markedly increased the total CIC and specifically increased

TABLE II

Effect of Immunostimulation on Serum IgG Isotypes, Autoantibodies, and IC

Murine strain background	Mode of immuno-stimulation	Total IgG*	Isotype distribution‡			Anti-single-stranded DNA binding	gp70 IC	Total CIC
			IgG1	IgG2a	IgG2b			
		mg/ml				%	*μg/ml*	*μg/ml*§
MRL/n	*lpr*	34	56	38	6	88 ± 21	16 ± 3	>400
	LPS	14	36	21	43	49 ± 12	23 ± 6	70 ± 22
	None	8	50	32	18	18 ± 10	5 ± 2	12 ± 2
C57BL/6	*lpr* + LPS	33	30	24	48	59 ± 21	11 ± 21	180 ± 112
	lpr	19	50	39	19	36 ± 10	3 ± 1	25 ± 40
	LPS	12	22	7	71	41 ± 15	3 ± 2	35 ± 20
	None	5	50	24	26	6 ± 4	<1	<4
C3H/HeJ	*lpr* + LPS	18	44	36	19	46 ± 16	2 ± 1	22 ± 20
	lpr	17	41	39	18	42 ± 16	2 ± 1	20 ± 16
	LPS	7	41	34	25	5 ± 4	<1	<4
	None	6	47	33	22	6 ± 3	<1	<4

Determinations were done in 5-mo-old MRL-*lpr* and MRL/n mice treated with LPS, 7-mo-old B6-*lpr* given LPS, and C3H-*lpr* given LPS, and in 10-mo-old B6-*lpr*, B6 given LPS, C3H-*lpr*, and C3H/HeJ mice given LPS. Values represent averages obtained with 15 mice per group.

* Sum of all three IgG subclasses measured by radial immunodiffusion.

‡ Values expressed as percent of total IgG.

§ Micrograms aggregated mouse gamma globulin equivalents per milliliter.

TABLE III

Effect of Immunostimulation on IgM RF and Their IgG Subclass Specificities

Murine strain background	Mode of immu-nostimulation	Total IgM	Total IgM RF	IgM RF reacting with IgG subclasses*			
				IgG1	IgG2a	IgG2b	IgG3
		μg/μl	*μg/ml*	*μg/ml*			
MRL/n	*lpr*	286	48	18	45	19	16
	LPS	257	34	33	16	16	17
	None	160	10	6	9	6	6
C57BL/6	*lpr* + LPS	477	150	71	55	55	73
	lpr	590	370	310	150	27	205
	LPS	350	45	42	19	20	25
	None	120	5	5	5	4	5
C3H/HeJ	*lpr* + LPS	500	230	167	88	54	86
	lpr	480	270	197	87	66	95
	LPS	140	5	5	3	4	5
	None	120	4	5	4	4	5

Determinations were done in 5-mo-old MRL-*lpr* and MRL/n mice given LPS, 7-mo-old B6-*lpr* given LPS, and C3H-*lpr* given LPS, and in 10-mo-old B6-*lpr*, B6 given LPS, C3H-*lpr* and C3H/HeJ mice given LPS. Values represent averages obtained with 15 mice per group.

* Values >20 μg/ml were considered positive for IgM RF activity, since such levels are not found in unmanipulated normal mice and other SLE mice lacking the *lpr* gene.

levels of retroviral glycoprotein 70 immune complexes (gp 70 IC), whereas LPS induced smaller increases (Table II). In the other two strains, each immunostimulator raised the levels of these two complexes somewhat above unmanipulated controls. In contrast, B6-*lpr* mice subjected to prolonged LPS stimulation showed significant CIC increases.

Anti–single-stranded DNA autoantibodies in all three *lpr* strains were increased over non-*lpr* controls, the highest in lupus-background mice. LPS increased these autoantibodies in the two LPS-responder strains, but not in

nonresponder C3H/HeJ. In MRL-*lpr* mice, most antibodies (70%) were IgG1 and IgG2a, while they were mostly IgM in young, normal-background *lpr* and LPS-treated non-*lpr* mice. Analysis of 5–10 sera from young and old *lpr* mice revealed that the switch from IgM to IgG anti–single-stranded DNA autoantibodies occurred in 4–5-mo-old MRL-*lpr* mice, but was delayed to 10–12 mo of age in normal-background *lpr* mice (data not shown).

Total IgM and IgM rheumatoid factors (RF) increased in MRL-*lpr* homozygous or normal genetic background mice, and in LPS-responding mice. The *lpr*-induced IgM RF were higher in B6-*lpr* and C3H-*lpr* than in MRL-*lpr* mice (Table III). MRL-*lpr* RF represented 23% of the total IgM; B6-*lpr* and C3H-*lpr* RF comprised 62 and 59% of their total IgM, respectively. Interestingly, chronic LPS treatment of B6-*lpr* mice reduced, rather than increased, IgM RF. MRL-*lpr* IgM RF exhibited higher reactivity with the IgG2a substrate, whereas B6-*lpr* and C3H-*lpr* IgM RF had higher anti-IgG1 activity. In the two responder strains, LPS induced polyclonal IgM RF that reacted primarily with IgG1.

Discussion

The main findings of this study are: (*a*) Genetic background influences the specificities and isotypes of autoimmune responses and the distribution and severity of immunopathology caused by endogenous (*lpr* gene) and/or exogenous immunostimulating factors (LPS), and (*b*) Synergistic action of multiple immunostimulators can overcome the previously observed (2–5) "resistance" of normal mice to the induction of significant autoimmune disease.

While the *lpr* gene accelerates latent GN and arthritis in SLE-prone MRL/n mice, LPS accelerates GN only. Differences in immunopathology induced by two accelerators acting on the same genetic background might be explained by differences in the cell types stimulated and in the fine characteristics of the resulting immunologic responses. Thus, while the *lpr* gene promotes Thy-1$^+$, Lyt-1$^+$ cell proliferation with enhanced helper activities (7), LPS directly stimulates B cells. Not surprisingly, the hypergammaglobulinemia and specificities of some induced autoantibodies differed. The increased IgG induced by *lpr* was primarily IgG1 and IgG2a, due to their strong T cell dependency (8). In contrast, LPS induced primarily IgG2b. While most RF induced by *lpr* in the MRL/n mice were anti-IgG2a, those stimulated by LPS were almost exclusively anti-IgG1. Complementarity of anti-IgG2a RF with the abundant IgG2a autoantibodies in *lpr* homozygous, lupus-background mice (9) should increase the amount and size of CIC, possibly enhancing their pathogenicity. In contrast, little complementation could occur between RF and autoantibodies in LPS-treated MRL/n mice and, therefore, the complexes formed may be less pathogenic. Moreover, proliferating T cells in MRL-*lpr* mice might have arthritogenic activities and/or, the increased Ia expression detected (10) in macrophages of MRL-*lpr* mice might allow better self-antigen presentation to the T cells infiltrating the synovium, thereby promoting tissue destruction.

In contrast to lupus-prone mice, introduction of *lpr* or LPS into normal-background mice failed to cause significant immunopathology. This was despite the induction of autoantibodies, as previously reported (4, 11). Higher amounts of autoantibodies and IC, and the earlier switch of autoantibodies from IgM to

IgG may account for the severe immunopathology in lupus-prone, but not in normal mice. It is likely that activated B cells in lupus-background mice (7, 12) respond better to proliferation and differentiation factors secreted by the *lpr*-induced abnormal T cells, thereby allowing a higher number of switching events. The lower levels of IgM RF in MRL-*lpr* mice (vs. normal-background *lpr/lpr* mice) may be caused by the removal of MRL-*lpr* RF from the circulation after coupling with complementary IgG2a autoantibodies, which should not occur as efficiently with the predominantly anti-IgG1 RF of normal-background *lpr* homozygotes. This possibility is supported by the observations that ~60% of the total IgM in normal-background *lpr* homozygous mice have RF activity, vs. 23% in MRL-*lpr* mice.

The novel finding is that two simultaneously administered immunostimulators can overcome the resistance shown by B6 mice to induction of significant autoimmune disease by either stimulator alone. LPS-activated B6-*lpr* B cells may have an increased ability to receive and respond to *lpr*-induced antigen-nonspecific differentiation factors, which allows increased polyclonal Ig and autoantibody production above threshold levels. In lupus-background (MRL/n) mice, the apparently increased numbers of spontaneously activated B cells (9, 12) allows them to respond to *lpr* stimulation alone to attain their pathogenic potentials.

Previous studies (13, 14) indicate that in vivo and in vitro lymphocytes from normal mice secrete autoantibodies spontaneously or after mitogenic stimulation. Idiotypes expressed on autoantibodies from lupus mice are also expressed in normal mice (15) and, conversely, idiotypes of autoantibodies from normal mice can be found in lupus mice (16). Despite this, the idiotype similarities may be only partial, or caused by parallel sets of antibodies without specificities for autoantigens (15), and do not necessarily include pathogenic idiotypes. Our findings indicate that most of the autoantibody variable region gene repertoire is represented in normal-background mice, but needs an appropriate stimulus or combinations of stimuli to be fully expressed. These findings complement recent protein sequencing (17) and molecular cloning (18, 19) experiments which suggest that the structural genetic elements of autoantibodies from lupus mice are closely related to those encoding antibodies to exogenous antigens in normal mice.

Summary

Either of two immunostimulating factors (*lpr*, lipopolysaccharide) enhanced the pathogenic autoimmune responses of MRL/n mice, but the serologic and immunopathologic characteristics differed. In contrast, either factor, acting alone, caused minimal immunopathology in normal mice, despite autoantibody induction. Combined immunostimulation, however, caused fatal glomerulonephritis in normal-background C57BL/6 mice. These results show the profound influence of the background genome on the effects of immunostimulating agents, and show that resistance to autoimmune disease in immunologically normal mice is not absolute.

References

1. Murphy, E. D. 1981. Lymphoproliferation (*lpr*) and other single-locus models for murine lupus. *In* Immunologic Defects in Laboratory Animals. M. E. Gershwin and B. Merchant, editors. Plenum Publishing Co., New York. 143–171.

2. Tonietti, G., M. B. A. Oldstone, and F. J. Dixon. 1970. The effect of induced chronic viral infections on the immunologic disease of New Zealand mice. *J. Exp. Med.* 132:89.
3. Hang, L., J. H. Slack, C. Amundson, S. Izui, A. N. Theofilopoulos, and F. J. Dixon. 1983. Induction of murine autoimmune disease by chronic polyclonal B cell activation. *J. Exp. Med.* 157:874.
4. Warren, R. W., S. A. Caster, J. B. Roths, E. D. Murphy, and D. S. Pisetsky. 1984. The influence of the *lpr* gene on B cell activation: different antibody expression in *lpr* congenic mouse strains. *Clin. Immunol. Immunopathol.* 31:65.
5. Hang, L., A. N. Theofilopoulos, and F. J. Dixon. 1982. A spontaneous rheumatoid arthritis-like disease in MRL/1 mice. *J. Exp. Med.* 155:1690.
6. Theofilopoulos, A. N., R. S. Balderas, L. M. Hang, and F. J. Dixon. 1983. Monoclonal IgM rheumatoid factor derived from arthritic MRL/Mp-*lpr/lpr* mice. *J. Exp. Med.* 158:901.
7. Prud'homme, G. J., C. L. Park, T. M. Fieser, R. Kofler, F. J. Dixon, and A. N. Theofilopoulos. 1983. Identification of a B cell differentiation factor(s) spontaneously produced by proliferating T cells in murine lupus strains of the *lpr/lpr* genotype. *J. Exp. Med.* 157:730.
8. Isakson, P. C., E. Pure, E. S. Vitetta, and P. H. Krammer. 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. *J. Exp. Med.* 155:734.
9. Slack, J. H., L. M. Hang, J. Barkley, R. J. Fulton, L. D'Hoostelaere, A. Robinson, and F. J. Dixon. 1984. Isotypes of spontaneous and mitogen-induced autoantibodies in SLE-prone mice. *J. Immunol.* 132:1271.
10. Kofler, R., R. D. Schreiber, F. J. Dixon, and A. N. Theofilopoulos. 1984. Macrophage I-A/I-E expression and macrophage-stimulating lymphokines in murine lupus. *Cell. Immunol.* 87:92.
11. Izui, S., V. E. Kelley, M. Kazushige, H. Yoshida, J. B. Roths, and E. D. Murphy. 1984. Induction of various autoantibodies by mutant gene *lpr* in several strains of mice. *J. Immunol.* 133:227.
12. Raveche, E. S., A. D. Steinberg, A. L. DeFranco, and J. H. Tjio. 1982. Cell cycle analysis of lymphocyte activation in normal and autoimmune strains of mice. *J. Immunol.* 129:1219.
13. Pisetsky, D. S., and S. A. Caster. 1982. The B cell repertoire for autoantibody: frequency of precursor cells for anti-DNA antibodies. *Cell. Immunol.* 72:294.
14. Fish, F., and M. Ziff. 1982. The *in vitro* and *in vivo* induction of anti-double-stranded DNA antibodies in normal and autoimmune mice. *J. Immunol.* 128:409.
15. Datta, S. K., B. D. Stollar, and R. S. Schwartz. 1983. Normal mice express idiotypes related to autoantibody idiotypes of lupus mice. *Proc. Natl. Acad. Sci. USA.* 80:2723.
16. Monier, J. C., J. Brochier, A. Moreira, C. Sault, and B. Roux. 1984. Generation of hybridoma antibodies to double-stranded DNA from non-autoimmune BALB/c strain: studies on anti-idiotype. *Immunol. Lett.* 8:61.
17. Eilat, D., M. Hochberg, J. Pumphrey, and S. Rudikoff. 1984. Monoclonal antibodies to DNA and RNA from NZB/NZW F_1 mice: antigenic specificities and NH_2-terminal amino acid sequences. *J. Immunol.* 133:489.
18. Kofler, R., D. J. Noonan, D. E. Levy, M. C. Wilson, N. P. H. Møller, F. J. Dixon, and A. N. Theofilopoulos. 1985. Genetic elements utilized for a murine lupus anti-DNA autoantibody are closely related to those for antibodies to exogenous antigens. *J. Exp. Med.* 161:in press.
19. Diamond, B., and M. D. Scharff. 1984. Somatic mutation of the T15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. Natl. Acad. Sci. USA.* 81:5841.