

MURINE SERUM GLYCOPROTEIN gp70 BEHAVES AS AN ACUTE PHASE REACTANT*

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Relatively large amounts of a glycoprotein, designated gp70 because of its molecular weight of 70,000 and its structural and immunologic similarity to retroviral envelope protein, circulate in the blood of many murine strains free from any association with viral particles (1-5). Although one finds multiple immunologically and structurally related gp70 in all mice, the structure of this serum gp70 molecule most closely resembles, but is not identical by peptide fingerprint analysis, to the coat protein of xenotropic virus isolated only from NZB and NZB × NZW F₁ hybrid (NZB × W) mice (5-7).

The origin of serum gp70 and the mechanisms controlling its expression are still unclear. Although lymphoid cells express virion-free gp70 on their surfaces (8-10), these cells do not seem to be a major source for serum gp70, because neither thymectomy nor splenectomy affects serum levels of gp70 (11). A tissue or organ that is directly or indirectly responsive to testosterone was suggested as the origin of serum gp70 (11); however, no serological or immunohistochemical evidence indicates either production or subsequent release of gp70 into the circulating blood by any organ or tissue.

In recent experiments (12), a single injection of bacterial lipopolysaccharide (LPS)¹ greatly enhanced the expression of serum gp70 in murine strains with high baseline levels of gp70 (>10 µg/ml) such as NZB, NZB × W, NZW, BXSB, DBA/2, 129(G_{IX}⁺), but not in those with lower levels (<5 µg/ml) including C57BL/6, BALB/c, or 129(G_{IX}⁻). This increased expression of serum gp70 was unrelated to the activation of endogenous xenotropic virus or other viral genomes. Therefore, this experimental model seemed suitable for investigation of the cellular origin of serum gp70 and the control mechanism for its production. Our results indicate that serum gp70 behaves like an acute phase reactant (APR) and is synthesized mainly by hepatic cells, as are many other APR and serum proteins.

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¹ Abbreviations used in this paper: APR, acute phase reactant; BSA, bovine serum albumin; D-GalN, D-galactosamine; HGG, human gamma globulin; LPS, lipopolysaccharide; MuLV, murine leukemia virus; NPP, native protoplasmic polysaccharides; PBS, phosphate-buffered saline; PLP, periodate-lysine-paraformaldehyde; poly I-poly C, polyribonucleic-polyribocytidylic acid.

Materials and Methods

LPS. LPS purified from *Escherichia coli* 0111:B4 and a lipid A component from LPS of *Salmonella minnesota* R595 were generously provided by Dr. D. C. Morrison (Department of Microbiology, Emory University School of Medicine, Atlanta, Ga.). *E. coli* 0111:B4 LPS extracted by the phenol-water method (13) was further fractionated by Sepharose 4B column chromatography, and fraction II of this preparation was used (14). Native protoplasmic polysaccharides (NPP) (15), alkali-treated LPS (16) (both lipid A free), as well as untreated LPS from *E. coli* 0113 were kindly provided by Dr. J. A. Rudbach (Department of Microbiology, University of Montana, Missoula, Mont.). All LPS and NPP preparations were diluted to the desired concentrations with sterile saline and injected intraperitoneally in a final volume of 0.4 ml.

Mice. All mice used in this study were 6–12 wk old. NZB, NZB × W, BXSB, DBA/2, 129(G_{IX}⁺), 129(G_{IX}⁻), C57BL/6, and BALB/c mice were obtained from the mouse breeding colony at Scripps Clinic and Research Foundation. Mice were bled from the retroorbital plexus, and the resulting sera were stored at -20°C until use.

T Cell-depleted Mice and Radiation Chimeras. T cell-depleted mice were prepared as follows: 2 mo-old NZB × W male mice were thymectomized, subjected to whole body exposure of 950 rad of ¹³⁷Cs γ-rays (Gammacell 40, Atomic Energy of Canada Ltd., Ottawa, Can.), and reconstituted with 5 × 10⁶ syngeneic bone marrow cells treated with anti-Thy-1.2 sera (New England Nuclear, Boston, Mass.) and guinea pig complement (Pel-Freez Biologicals, Rogers, Ariz.). 16 d after reconstitution, recipients were injected with 25 μg of LPS i.p. and tested for serum gp70 responses. The effectiveness of T cell depletion was demonstrated by the marked impairment of antibody responses to the T dependent antigen, human gamma globulin (HGG), in T cell-depleted mice (24 ± 14 μg/ml) compared with unmanipulated mice (480 ± 35 μg/ml), and by the responsiveness of their spleen cells to 1 μg/ml of concanavalin A (stimulation index of T cell-depleted mice was 3.8 and of unmanipulated mice, 113.2).

Radiation chimeras were made as follows: after 129(G_{IX}⁺) and 129(G_{IX}⁻) mice received 900 rad of irradiation, we injected each substrain with 50 × 10⁶ spleen cells from the congenic counterpart. 1 mo later, 25 μg of LPS was administered i.p., after which the serum gp70 and IgM responses were monitored.

Assay of gp70. Concentrations of gp70 in sera and tissue extracts of test mice were determined by their capacity to inhibit the binding of goat antibody to feline leukemia virus to ¹²⁵I-labeled gp70 from Rauscher murine leukemia virus (MuLV). The details of this radioimmunoassay were described previously (17).

Assay of IgM and Anti-HGG. A solid-phase radioimmunoassay was performed to assess the serum levels of IgM. The same assay, but with human IgG used as the antigen, quantitated anti-HGG antibodies in sera after mice received an intravenous injection of 400 μg heat-aggregated HGG. These assays were performed as described (18, 19).

Quantitation of Haptoglobin. Concentrations of haptoglobin in murine serum were measured by radial immunodiffusion in agar with goat anti-human haptoglobin antiserum (N. L. Cappel Laboratories, Cochranville, Pa.) used according to the method of Mancini et al. (20). Results are expressed as a percentage of values from the pooled sera of 3-mo-old C57BL/6 male mice.

Chemical Reagents. Turpentine oil was obtained from American Scientific Products, Irvine, Calif. Polyribonucleic-acid-polyribocytidylic acid (poly I-poly C), colchicine and D-galactosamine (D-GalN) were purchased from Sigma Chemical Company, St. Louis, Mo. and dissolved in pyrogen-free saline immediately before use.

Preparation of Tissue Extracts. Samples weighing 0.06–0.2 gr from livers, kidneys, spleens, thymuses, and lungs of test mice were placed in tissue grinders and mixed with cold 0.01 M phosphate-buffered saline (PBS), pH 7.2, to yield a 10% homogenate (wt/vol). These tissue homogenates in an ice bath were sonicated with a Sonifier Cell Disrupter (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) and then centrifuged at 2,000 g for 15 min at 4°C. The supernatants were treated with Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) in a final concentration of 0.4% at 37°C for 15 min, followed by three cycles of freezing and thawing and centrifugation at 2,000 g for 15 min at 4°C. The final supernatants were used for the quantitation of tissue gp70.

Determination of gp70 Content in Tissues. Blood content in each tissue extract was quantitated

by using ^{131}I -labeled bovine serum albumin (BSA; Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) as a volume marker according to the modified procedure of Mathison and Ulevitch (21). Briefly, 0.2–0.3 mg of ^{131}I -BSA with a specific activity of 1.5–2.5 $\mu\text{Ci}/\mu\text{g}$ was injected intravenously into NZB \times W female mice, whose sera were collected 3 h later. The radioactivity of sera with approximately 15–20 $\mu\text{Ci}/\text{ml}$ was >90% precipitable in 10% trichloroacetic acid. 50–100- μl samples containing 1×10^6 – 2×10^6 cpm of ^{131}I -BSA were then injected intravenously into 2-mo-old NZB \times W female mice 5 min before killing. On the basis of counts per minute per ml of tissue extract and per ml of blood, the blood content in various tissue extracts was calculated. From the measurement of gp70 content in tissue extracts and in blood, the amounts of gp70 present in tissue were computed.

Fluorescence Microscopy for Detection of gp70 in Livers. To localize gp70 in livers of test mice, each liver was perfused with 0.05 M PBS, pH 7.2, and with the periodate-lysine-paraformaldehyde (PLP) solution as described by McLean and Nakane (22). Then, 2–4 mm liver fragments were fixed with PLP for 3 h at 4°C and washed, first with 7% sucrose in PBS, then with 15% sucrose in PBS, and finally with PBS containing 25% sucrose and 20% glycerol. The fixed liver samples were embedded in Ames OCT compound, quick frozen, and sectioned into 4- μm slices on a cryostat at –35°C. To demonstrate gp70 in this tissue, sections were incubated with the IgG fraction of goat anti-Rauscher MuLV gp70 (the gift of Dr. J. H. Elder, Scripps Clinic and Research Foundation) or normal goat IgG for 30 min at room temperature. After being washed, fluorescein isothiocyanate-conjugated rabbit anti-goat IgG (N. L. Cappel Laboratories) was applied for 30 min at room temperature. The specificity of fluorescence was controlled by absorbing the goat anti-Rauscher MuLV gp70 with Rauscher MuLV. For this purpose, anti-gp70 IgG (0.5 ml of a 6 mg/ml solution) was incubated with 3 mg Rauscher MuLV for 30 min at 37°C, followed by centrifugation at 100,000 *g* for 60 min at 4°C. The resulting supernatant was used as absorbed antibodies.

Results

Induction of High Serum Levels of gp70 in Mice by LPS. In 2-mo-old NZB female mice whose baseline levels of serum gp70 were $46 \pm 12 \mu\text{g}/\text{ml}$, the injection of 25 μg i.p. of LPS from *E. coli* 0111:B4 caused an increase of gp70 to $376 \pm 73 \mu\text{g}/\text{ml}$ within 24 h. This gp70 response was dose related (Fig. 1). Serum gp70 concentrations increased significantly after injection of 25 ng of LPS ($137 \pm 27 \mu\text{g}/\text{ml}$) and reached near-maximal responses with 25 μg of LPS in NZB mice. Results were similar in strains with high baseline levels of serum gp70 (>10 $\mu\text{g}/\text{ml}$) such as NZB \times W, BXSB, DBA/2, and 129(G_{1x}⁺) mice. However, murine strains with low serum gp70 (<5 $\mu\text{g}/\text{ml}$) such as C57BL/6 or BALB/c mice showed no increase in serum gp70 at any dose of LPS, as described previously (12).

To define that portion of the LPS molecule responsible for stimulating gp70 response, the lipid A fraction extracted from *S. minnesota* R595 LPS and lipid A-free preparations of *E. coli* 0113 LPS, such as NPP and alkali-treated LPS, were tested for gp70-producing capacity in NZB \times W mice. The injection of purified lipid A induced serum levels of gp70 about equal to those stimulated by the entire LPS molecule (Table I). In contrast, NPP and alkali-treated LPS preparations, both of which lack the lipid A component, induced only minimal activity.

Unrelatedness of Serum gp70 Induction by LPS and Activation of Lymphocytes. Our previous studies (12) demonstrated that the increase in serum gp70 after injection of LPS is not associated with activation of endogenous virus nor attributable to the cellular death caused by LPS, but results from active synthesis of gp70. Because thymocytes express retroviral gp70 on their surfaces (8–10), we now examined the possible participation of T cells in the serum gp70 response stimulated by LPS. After T cell-depleted NZB \times W mice (thymectomized and reconstituted with anti-Thy-1.2 and comple-

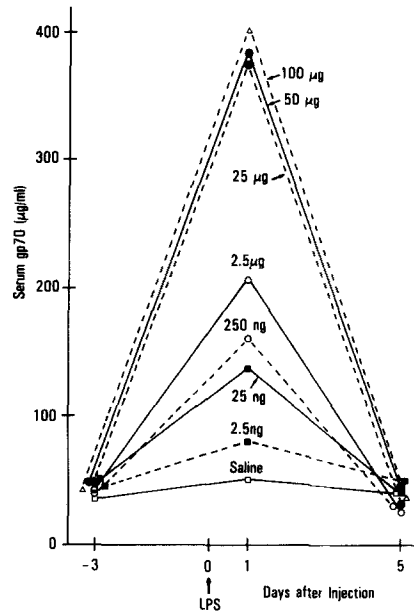


FIG. 1. Serum gp70 responses of 2-mo-old NZB female mice injected with various doses of LPS from *E. coli* 0111:B4. Each point represents a mean value from five mice.

TABLE I
Effect of Various Preparations of LPS on Serum gp70 Responses

LPS preparations*	Serum gp70	
	Day 0	Day 1
	<i>µg/ml</i>	
LPS	47.3 ± 12.9‡	280.7 ± 41.3
NPP	50.1 ± 3.8	85.5 ± 17.6
Alkali-treated LPS	51.5 ± 10.2	94.5 ± 39.6
Lipid A	44.7 ± 3.1	312.5 ± 99.8
Saline	48.2 ± 14.5	52.2 ± 6.7

* 50 µg of each LPS preparation was injected i.p. on day 0 into 2-mo-old NZB × W male mice. LPS, NPP, and alkali-treated LPS were purified from *E. coli* 0113 and lipid A from *S. minnesota* R595. Note that both NPP and alkali-treated LPS lacked lipid A.

‡ Mean ± 1 SD for five mice.

ment-treated syngeneic bone marrow cells) received 25 µg LPS i.p., their serum gp70 responses were compared with those of unmanipulated NZB × W mice. LPS increased serum gp70 levels from 61 ± 22 µg/ml to 336 ± 82 µg/ml in T cell-depleted mice. This increased response was only slightly lower than that in controls with normal numbers of T cells (from 62 ± 11 µg/ml to 590 ± 69 µg/ml), thus excluding a major role of T cells in serum gp70 responses induced by LPS.

LPS is a potent B cell activator (23), and the activation of B cells might account for the enhanced expression of gp70 on their surfaces (24, 25). Therefore, we examined the possible relationship between synthesis of serum gp70 promoted by LPS and the activation of B cells, measured as serum levels of polyclonal IgM antibodies. For this

purpose, 2-mo-old NZB \times W mice were lethally irradiated (1,000 rad), and 2 d later, were injected with 25 μ g of LPS. 1 d after injection of LPS, concentrations of serum gp70 were \sim 10 times as high as those before injection of LPS regardless of irradiation (Table II). However, 5 d after the injection of LPS, serum IgM levels doubled in unirradiated mice, but actually dropped in irradiated mice. Radiation alone had no significant effect on serum levels of gp70, but again somewhat decreased the concentrations of serum IgM.

To confirm that the activation of lymphocytes was unrelated to the LPS-induced serum gp70 response, this response was examined in radiation chimeras of 129(G_{IX}^+) and 129(G_{IX}^-) congenic mice. It had been shown that LPS enhances the expression of serum gp70 in the former high gp70 mice but not in the latter (12), which have much lower concentrations of gp70 in sera and on the surface of lymphocytes (2, 10, 12). Baseline levels of serum gp70 in irradiated 129(G_{IX}^+) or 129(G_{IX}^-) mice reconstituted with reciprocal spleen cells were comparable to those of untreated 129(G_{IX}^+) or 129(G_{IX}^-) mice, regardless of the donor's cell type (Table III). Unmanipulated G_{IX}^+ mice and G_{IX}^+ mice given G_{IX}^- spleen cells responded equally well to the injection of 25 μ g of LPS, developing high serum levels of gp70. However, little increase in serum

TABLE II
Serum gp70 and IgM Responses to LPS in Lethally Irradiated NZB \times W Mice

Treatment	Injection	gp70		IgM	
		Day 0	Day 1	Day 0	Day 5
		μ g/ml		μ g/ml	
Radiation*	LPS‡	46.2 \pm 5.2§	476.1 \pm 65.6	627 \pm 104	443 \pm 168
None	LPS	54.2 \pm 4.3	561.3 \pm 53.9	530 \pm 103	1,158 \pm 118
Radiation	Saline	47.1 \pm 15.8	46.1 \pm 3.2	632 \pm 75	442 \pm 35
None	Saline	48.4 \pm 5.2	54.1 \pm 7.6	664 \pm 28	586 \pm 61

* 2-mo-old NZB \times W male mice were irradiated with 1,000 rad 2 d before injection of LPS from *E. coli* 0111:B4.

‡ 25 μ g LPS was injected i.p. on day 0.

§ Mean \pm 1 SD for six mice.

TABLE III
Failure of G_{IX}^- Mice Reconstituted with G_{IX}^+ Spleen Cells to Produce Serum gp70 after Injection of LPS

Mice*	Serum gp70‡		Serum IgM‡		
	Day 0	Day 1	Day 0	Day 5	
		μ g/ml		μ g/ml	
I. G_{IX}^+ given G_{IX}^- cells	7.0 \pm 0.9§	65.6 \pm 13.5	253 \pm 77	946 \pm 196	
II. G_{IX}^+	8.3 \pm 1.3	58.4 \pm 15.4	180 \pm 42	808 \pm 245	
III. G_{IX}^- given G_{IX}^+ cells	1.1 \pm 0.3	2.2 \pm 1.1	200 \pm 36	1,035 \pm 252	
IV. G_{IX}^-	0.3 \pm 0.2	1.1 \pm 0.8	173 \pm 15	1,038 \pm 109	

* I. G_{IX}^+ mice reconstituted with G_{IX}^- spleen cells.

II. Unmanipulated G_{IX}^+ mice.

III. G_{IX}^- mice reconstituted with G_{IX}^+ mice.

IV. Unmanipulated G_{IX}^- mice.

‡ Serum levels of gp70 and IgM were determined 1 and 5 d, respectively, after injection of 25 μ g of LPS from *E. coli* 0111:B4.

§ Mean \pm 1 SD for 5-10 mice.

gp70 was seen in 129(G_{IX}^-) recipients reconstituted with G_{IX}^+ spleen cells or unmanipulated 129(G_{IX}^-) mice. It should be noted that a higher frequency of gp70-positive cells in the spleens of G_{IX}^- mice reconstituted with G_{IX}^+ cells than in G_{IX}^+ mice given G_{IX}^- cells revealed that spleen cells of both chimeras were of donor type. Further, sera of both groups of mice developed comparable amounts of polyclonal IgM antibodies as a result of B cell activation by LPS (Table III).

Serum gp70 as an APR. Because LPS is a potent inducer of APR (26–28), the kinetics of serum gp70 responses were compared with those of the APR, haptoglobin, in LPS-injected NZB \times W female mice. Serum levels of both gp70 and haptoglobin increased as early as 6 h after the injection and peaked at \sim 24 h, although gp70 levels returned to the preinjection levels more rapidly than those of haptoglobin (Fig. 2). At their peaks, gp70 and haptoglobin levels were \sim 10 times higher than those before injection of LPS.

The possible stimulation of serum gp70 synthesis by various inducers of APR was also investigated. NZB \times W male mice were injected intraperitoneally with 0.01 or 0.05 ml of turpentine oil or 100, 200, or 500 μ g of poly I-poly C. 1 d after the injection, dose-dependent increases of serum gp70 and of haptoglobin were observed in the animals injected with turpentine oil or poly I-poly C (Table IV). 0.05 ml of turpentine oil and 500 μ g of poly I-poly C induced the production of haptoglobin and serum gp70 as effectively as 25 μ g of LPS. This finding was confirmed in DBA/2 and BXSB mice, which are natively high in serum gp70 concentrations (data not shown). Table IV also shows that although LPS stimulated the production of serum IgM as a result of B cell activation, neither turpentine oil nor poly I-poly C elevated the serum levels of IgM, ruling out possible contamination by LPS in preparations of turpentine oil and poly I-poly C.

Tissue Origin of Serum gp70 Induced by LPS. Because, in the foregoing experiments, the induction of serum gp70 by LPS was apparently unrelated to the activation of

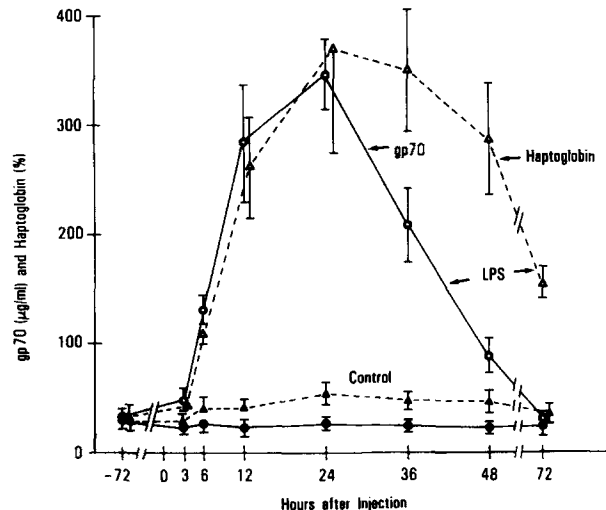


FIG. 2. Serum gp70 and haptoglobin responses of 2-mo-old NZB \times W female mice to injection of 25 μ g of LPS from *E. coli* 0111:B4. Each point represents mean from five mice. Vertical bars represent the limits of 1 SD. O, gp70 (LPS); ●, gp70 (control); Δ , haptoglobin (LPS); \blacktriangle , haptoglobin (control).

TABLE IV
Serum gp70, Haptoglobin, and IgM Responses after Injection of Inducers of APR

Inducers*	gp70‡	Haptoglobin‡	IgM‡
	$\mu\text{g/ml}$	%	$\mu\text{g/ml}$
LPS 25 μg	590 \pm 69§	421 \pm 52	1,640 \pm 305
Turpentine oil 0.01 ml	172 \pm 34	503 \pm 137	644 \pm 87
	0.05 ml	413 \pm 38	533 \pm 183
Poly I-poly C 100 μg	322 \pm 84	257 \pm 46	456 \pm 133
	200 μg	426 \pm 58	507 \pm 86
	500 μg	543 \pm 122	438 \pm 29
Saline	63 \pm 6	119 \pm 32	604 \pm 101

* Inducers of APR were injected i.p. on day 0 into 2-3-mo-old NZB \times W male mice.

‡ Serum levels of gp70 and haptoglobin were determined 1 d after injection and those of IgM, 5 d after.

§ Mean of five to eight mice \pm 1 SD.

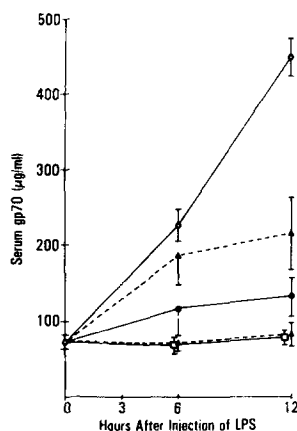


FIG. 3. The effect of colchicine on the serum gp70 response induced by 25 μg of LPS from *E. coli* 0111:B4. The different doses of colchicine were injected 3 h after injection of LPS. Each point represents mean \pm 1 SD for five mice. ○, LPS alone; △, LPS + 15 μg colchicine; ●, LPS + 100 μg colchicine; □, 100 μg colchicine; ▲, saline.

lymphocytes, we sought this glycoprotein's site of origin in the liver, a major source of many other serum proteins. The effect of colchicine, an inhibitor of the plasma protein's secretion from the liver, on serum gp70 responses to LPS was examined in NZB \times W male mice. Inoculation of 25 μg LPS followed 3 h later by 100 μg of colchicine yielded greatly suppressed rises in serum levels of gp70 compared to those induced by LPS alone (Fig. 3). 12 h after injection of LPS in colchicine-treated mice, serum levels of gp70 were less than one third (133 \pm 25 $\mu\text{g/ml}$) of those in mice receiving LPS alone (449 \pm 25 $\mu\text{g/ml}$). Less, but still significant suppression was observed in mice injected with 15 μg colchicine (215 \pm 47 $\mu\text{g/ml}$). Colchicine alone did not significantly change baseline levels of gp70 in serum.

With this experimental system, the quantity of gp70 in various organs was then determined in NZB × W female mice. The first group of mice were injected with 25 µg LPS alone, the second with 25 µg LPS and 3 h later 100 µg of colchicine, the third with colchicine alone, and the fourth with saline alone (controls). gp70 was quantitated in livers, kidneys, spleens, thymuses, and lungs as well as in sera 9 h after the injection of LPS. The amounts of gp70 recoverable in liver extracts were 3.3 times higher in mice receiving LPS alone than in saline-injected controls (Table V). However, other tissue extracts did not show this remarkable increase in the amounts of gp70. The subsequent injection of colchicine in LPS-treated mice further increased the amounts of gp70 in their livers, but not in any other tissues tested. The liver extracts from such mice contained 2.4 times as much gp70 as those from the animals injected with LPS alone, or 7.8 times as much as those from saline controls, in striking contrast to the blunted serum gp70 responses in colchicine-treated mice. Livers from animals treated with colchicine alone contained 2.3 times higher concentrations of gp70 than saline-injected controls. It was noteworthy that levels of gp70 in sera and livers never increased after injection of LPS administered simultaneously with D-GalN, an inhibitor of protein synthesis (Table VI).

To further identify the cellular origin of gp70, livers from NZB × W mice were analyzed immunohistochemically. Antiserum specific for Rauscher MuLV gp70

TABLE V
Accumulation of gp70 in Livers from Mice Injected with LPS and Colchicine

Treatment	Serum gp70*	Tissue gp70*				
		Liver	Kidney	Spleen	Thymus	Lung
	µg/ml			µg/organ		
LPS‡	329 ± 48	50 ± 8	29 ± 9	14 ± 1	12 ± 3	11 ± 2
LPS + Colchicine§	172 ± 30	118 ± 23	33 ± 5	16 ± 1	13 ± 2	11 ± 2
Colchicine	47 ± 14	34 ± 5	28 ± 4	11 ± 1	10 ± 4	11 ± 2
Saline	43 ± 8	15 ± 4	22 ± 5	14 ± 3	10 ± 1	9 ± 2

* Amounts of gp70 in sera and in tissues were determined 9 h after the injection of LPS. Mean ± 1 SD for seven mice.

‡ 25 µg of LPS from *E. coli* 0111:B4 was injected i.p. into 2-mo-old NZB × W female mice.

§ 100 µg of colchicine was injected i.p. 3 h after the injection of LPS.

TABLE VI
Suppression of LPS-Induced gp70 Response in Serum and Liver by D-GalN

Treatment	gp70*	
	Serum	Liver
	µg/ml	µg/organ
LPS‡	343.5 ± 55.8	66.9 ± 12.9
LPS + D-GalN§	25.4 ± 6.9	18.9 ± 2.9
D-GalN	18.3 ± 1.6	16.5 ± 1.8
Saline	36.4 ± 7.5	20.2 ± 3.3

* Amounts of gp70 in sera and livers were determined 9 h after injection of LPS. Mean ± 1 SD for five mice.

‡ 25 µg of LPS from *E. coli* 0111:B4 was injected i.p. into 2-mo-old NZB × W female mice.

§ 7.5 µg of D-GalN was injected i.p. simultaneously with LPS.

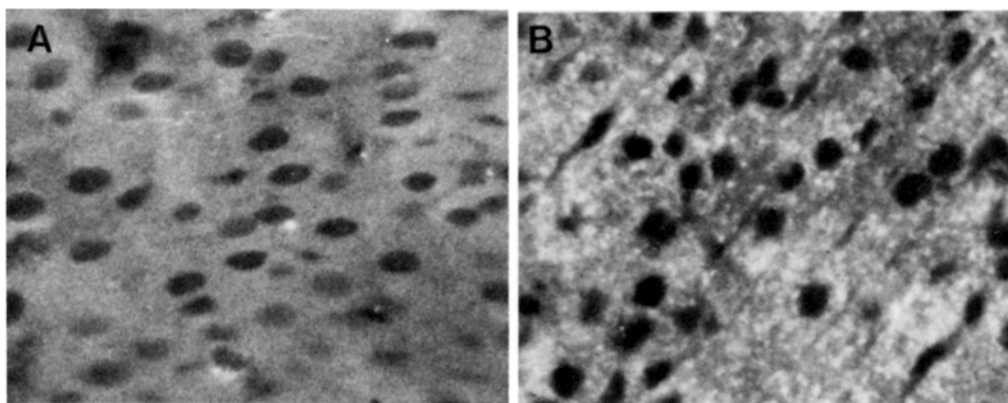


FIG. 4. Demonstration of gp70 in liver from NZB \times W mice injected with LPS and colchicine by indirect immunofluorescence using goat anti-Rauscher MuLV gp70. (A) Liver specimen from uninjected control mice. No specific staining for gp70 is seen on these hepatocytes. (B) Section of liver from mice injected with LPS and colchicine. Colchicine (100 μ g) was injected 3 h after the injection of LPS (25 μ g) and liver was taken 9 h after LPS injection. Granular staining indicates accumulation of gp70 in cytoplasm of hepatocytes. \times 420.

scarcely stained hepatocytes of untreated mice examined by indirect immunofluorescence (Fig. 4A). However, livers of mice obtained 6 h after administration of LPS began to show a fine granular staining of gp70 in most hepatocytes and in some sinusoidal margins, and at 9 h the distribution of gp70 was more extensive. Compared with mice injected with LPS alone, livers from mice receiving both LPS and colchicine showed more intense, coarse granular staining of gp70 in the cytoplasm of virtually all the hepatocytes without appreciable staining along the sinusoids (Fig. 4B). Liver sections from mice treated with colchicine alone also had increased numbers of gp70-positive hepatocytes. After absorption of antisera to Rauscher MuLV gp70 with Rauscher MuLV, no positive staining appeared. Liver sections from BALB/c or C57BL/6 mice, which do not produce gp70 after injection of LPS (12), showed no such specific staining, even after treatment with LPS and colchicine.

Discussion

Using an experimental model in which the expression of serum gp70 is greatly enhanced after the injection of LPS (12), we have demonstrated that this gp70 behaves as an APR and is mainly synthesized by hepatic cells. Apparently, this glycoprotein is significantly involved as an autoantigen in the pathogenesis of murine systemic lupus erythematosus (7, 17, 29–31).

Lymphocytes have been suggested as the major source of serum gp70 (10). Indeed, lymphocytes express surface gp70 molecules with characteristics similar to serum gp70 (10, 32), and enhanced production of serum gp70 induced by LPS is seen only in murine strains whose thymocytes are positive for G_{1x} antigen (12), a type-specific determinant of the gp70 molecule (2, 9, 10, 33). However, our results refute this possibility. First, T cell-depleted mice produce as much serum gp70 as mice with normal numbers of T cells after the injection of LPS. Second, the production of gp70 is independent of the activation of B cells, because lethal irradiation of mice preceding the injection of LPS completely abolishes the activation of B cells, but has no effect

on the serum gp70 response. Further, gp70 production is stimulated by such inducers of APR as turpentine oil or poly I-poly C without significant formation of polyclonal IgM antibodies. Finally, 129(G_{IX}⁺) mice produce gp70 after stimulation with LPS, even after irradiation and reconstitution with spleen cells from G_{IX}⁻ mice. To the contrary, 129(G_{IX}⁻) mice either unmanipulated or replenished with G_{IX}⁺ spleen cells develop no serum gp70 responses. This experiment with radiation chimeras clearly indicates that the ability to generate a serum gp70 response is not dependent on the genotype of spleen cells, but rather related to that of nonlymphoid tissues. Of note, both chimeras developed equally high levels of polyclonal antibodies as a result of B cell activation. These results are consistent with a previous report (11) that neither thymectomy, splenectomy, nor exchange of hematopoietic tissues between 129(G_{IX}⁺) and 129(G_{IX}⁻) mice affected levels of serum gp70 in either strain. All these findings exclude lymphoid cells as a major source of serum gp70.

Instead, our results strongly suggest the liver as the major site of serum gp70 production. The experimental observations that support this conclusion are: First, amounts of gp70 present in liver extracts of LPS-injected mice were more than three times higher than in uninjected controls, whereas no such increase appeared in other tissues including the lung, kidney, spleen, or thymus. Second, the injection of colchicine, which is a known inhibitor of protein release from hepatic cells (34, 35), greatly suppressed the serum gp70 response. Accordingly, increased amounts of gp70 were recoverable from liver extracts but not other tissue extracts, indicating the accumulation of newly synthesized gp70 in hepatic cells as colchicine inhibited its release. Third, D-GalN, an inhibitor of protein synthesis (36, 37), completely prevented increase of gp70 in the liver or serum after the injection of LPS. Finally, immunohistochemical study more directly showed the presence of gp70 as fine granules in the cytoplasm of hepatic parenchymal cells from mice receiving LPS. Subsequent treatment with colchicine intensified this specific staining by gp70 in almost all the hepatocytes. In all probability, hepatic cells are the major source of the gp70 that eventually enters the circulation. This conclusion is compatible with the fact that the concentration of gp70 in sera is generally 20–100% higher in male mice than females (11, 12, 17). The liver is a known source of several proteins whose serum concentrations are associated markedly with the host's sex depending on androgenic hormones (38).

A particularly significant observation in this research is that serum gp70 behaves like an APR. Not only LPS but also other inducers of APR, such as turpentine oil or poly I-poly C (39, 40), enhanced the synthesis of serum gp70. Notably, neither turpentine oil nor poly I-poly C activated B cells to produce polyclonal antibodies, ruling out possible contamination with LPS in these preparations. Further, the kinetics of this gp70 response were essentially identical to those of such APR as haptoglobin, serum amyloid A protein, or C-reactive protein (26–28). Finally, we recently demonstrated that restriction of calorie intake in NZB × W mice reduced serum levels of the APR, haptoglobin, as well as gp70, but not that of albumin (41). Consequently, serum gp70 has the qualities of an APR, and its expression is controlled by a mechanism similar to that for other APR. Hepatic cells undoubtedly synthesize gp70 and release it into the circulation as they do other APR (26, 28, 39, 42, 43).

In view of (a) the similarity in expression of serum gp70 and a number of other proteins that are products of the host genome, (b) the failure of other retroviral gene (even *env* gene) products to be expressed along with serum gp70, and (c) the slight but

demonstrable differences between serum gp70 and the known products of any retroviral *env* gene, it seems that this molecule behaves as a host gene product.

Summary

A single intraperitoneal injection of bacterial lipopolysaccharide (LPS) or its lipid A component induced high levels of glycoprotein, gp70, in sera of several strains of mice within 24 h. This serum gp70 response induced by LPS was independent of the activation of B cells and the presence of T cells. However, serological and immunohistochemical studies demonstrated the production of gp70 by hepatic parenchymal cells and its subsequent release into the circulating blood.

The expression of gp70 in the serum was enhanced not only by LPS but also other inducers of acute phase reactants (APR) such as turpentine oil or polyribonucleic acid. Further, the serum gp70 response was kinetically identical to those of APR. These results strongly suggest that (a) the liver may be the major source for serum gp70, (b) serum gp70 behaves like an APR, (c) its expression may be controlled by a mechanism similar to that for other APR, and (d) this glycoprotein apparently behaves as a normal host constituent and not a product of a viral genome.

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