

**SOURCE AND HORMONE-DEPENDENCE OF G_{IX}-gp70
IN MOUSE SERUM***

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The major envelope glycoprotein of murine C-type virus is designated gp70 because of its mol wt of 70,000 daltons (1). One member of this family of gp70 molecules, G_{IX}-gp70, is distinguished by the type-specific antigen G_{IX}, which is recognized by the complement-dependent cytotoxicity assay on thymocytes of G_{IX}⁺ mouse strains (2-4). In certain G_{IX}⁺ mice, of which strain 129 has been most studied, expression of G_{IX}-gp70 is a Mendelian trait and not associated with the production of demonstrable virions (2).

In 129 mice, G_{IX}-gp70 occurs in the serum (s-G_{IX}-gp70) and in various epithelia associated with the digestive tract (3, 5). G_{IX}-gp70 is also present in the epithelial lining of the male reproductive tract, which presumably accounts for the large amount of G_{IX}-gp70 in seminal fluid (5). In mice of the congenic strain 129-G_{IX}⁻ (genotype *Gv-1*⁻), G_{IX}-gp70 is evidently absent, indicating that the genetic locus *Gv-1* regulates the expression of G_{IX}-gp70 at all sites where it can be demonstrated in 129 mice (genotype *Gv-1*⁺) (6).

The following study was undertaken initially to find the tissue origin of the G_{IX}-gp70 which is free in the serum of 129 mice. The various known G_{IX}⁺ tissues mentioned above were ruled out as major sources of s-G_{IX}-gp70, and it was found that s-G_{IX}-gp70 levels are highly hormone-dependent.

Materials and Methods

Mice. All mice were from our colonies at Sloan-Kettering Institute.

Estimation of G_{IX}-gp70 in Serum (s-G_{IX}-gp70). Levels of s-G_{IX}-gp70 were measured by the capacity of serum samples (antigen) to inhibit the cytotoxic activity of G_{IX} antiserum [(W/Fu × BN)F₁ rat anti-murine leukemia virus-induced W/Fu rat leukemia (C58NT)D (2)] for C57BL/6-G_{IX}⁺ thymocytes as described in detail elsewhere (3). Briefly, all mouse serum samples to be tested for s-G_{IX}-gp70 were preabsorbed with BALB/c thymocytes to remove naturally occurring antibody to thymocytes (7). Equal volumes (20 μl) of antigen (the serum samples; serially diluted) and G_{IX} antiserum (dilution predetermined; usually 1:200) were mixed and incubated for 30 min on ice. Then 50 μl of a suspension of C57BL/6-G_{IX}⁺ thymocytes (5 × 10⁶/ml) was added to the mixture, and incubation continued for 30 min on ice. The cells were then washed once with medium 199, resuspended in 100 μl of diluted rabbit serum (pretested complement source), incubated for 30 min at 37°C, and counted in the presence of trypan blue (percent dead cells = "a"). In controls, medium 199 was substituted for the serum samples

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(percent dead cells, control = "b"). The resulting estimate of s-G_{IX}-gp70 was expressed as an inhibition index, (b - a)/b.

Radiation Chimeras. Mice were lethally irradiated (850 rads; Gammacell 40, Atomic Energy of Canada) and restored with 1×10^8 mixed bone marrow and spleen cells intravenously.

Results

Thymectomy and Splenectomy. Excision of neither the thymus (Table I; groups 2 and 8) nor the spleen (groups 3 and 9) from either sex, influenced the levels of s-G_{IX}-gp70, measured 6 wk later (normal controls; groups 1 and 7). These measurements were unchanged at 12 wk after thymectomy or splenectomy (data not shown).

Serum G_{IX}-gp70 in Radiation Chimeras. We established further that s-G_{IX}-gp70 does not originate in demonstrable quantity from thymocytes or from any hematopoietic cell in the following manner: chimeras were made by lethally irradiating 129-G_{IX}⁻ congenic mice and restoring them with bone marrow and spleen cells of 129 donors. The serum of these chimeras, tested 3 wk later (data not shown) and 6 wk later (Table I), contained no measurable G_{IX} antigen (group 10). Also, the level of s-G_{IX}-gp70 in lethally irradiated 129 mice restored with bone marrow and spleen cells from 129-G_{IX}⁻ donors was as high as in normal 129 mice of the same age and sex (group 11). The thymocytes of both types of chimera were of donor G_{IX} type, implying virtually total restoration of hemopoiesis by donor cells.

Sex Differences in Levels of s-G_{IX}-gp70. The amount of s-G_{IX}-gp70 is higher in the serum of 129 males than of 129 females (Table I and Fig. 1) and the level rises with age in both sexes (Fig. 1). Titrations of antigen (s-G_{IX}-gp70) in the inhibition assay

TABLE I
Levels of G_{IX}-gp70 in the Serum of Mice* of Various Experimental Groups

Group	Sex (number of mice tested)	Treatment	Serum G _{IX} -gp70 (Inhibition index)	
			After 6 wk (± SEM)	After 6 wk, and then 10 day's tes- tosterone (± SEM)
First set of experiments‡				
1	♂ (5)	None (age-matched controls)	0.68 (± 0.02)	
2	♂ (5)	Thymectomy	0.85 (± 0.06)	
3	♂ (4)	Splenectomy	0.72 (± 0.04)	
4	♂ (4)	Bilateral excision of testis, epididymis, and vas deferens	0.19 (± 0.01)	
5	♂ (5)	Unilateral excision of testis, epididymis, and vas deferens	0.73 (± 0.08)	
6	♂ (6)	Thymectomy and bilateral excision of testis, epididymis, and vas deferens	0.22 (± 0.03)	
7	♀ (5)	None (age-matched controls)	0.23 (± 0.09)	
8	♀ (5)	Thymectomy	0.38 (± 0.10)	
9	♀ (5)	Splenectomy	0.25 (± 0.01)	
Second set of experiments‡				
10	♂ (4) (129-G _{IX} ⁻)	Lethal irradiation; restored with 129 ♀ marrow and spleen cells	0.00 (± 0.00)	
11	♂ (4) (129)	Lethal irradiation; restored with 129-G _{IX} ⁻ ♀ marrow and spleen cells	0.51 (± 0.08)	
Third set of experiments‡				
12	♂ (4)	None (age-matched controls)	0.81 (± 0.06)	0.77 (± 0.08)
13	♂ (5)	Bilateral excision of testis, epididymis, and vas deferens	0.21 (± 0.01)	0.75 (± 0.05)
14	♂ (5)	Bilateral excision of testis	0.25 (± 0.06)	0.77 (± 0.02)
15	♂ (4)	Bilateral excision of epididymis, and vas deferens	0.67 (± 0.05)	0.83 (± 0.06)
16	♀ (5)	None (age-matched controls)	0.22 (± 0.05)	0.55 (± 0.17)

* All of strain 129, except the 129-G_{IX}⁻ congenic mice of groups 10 and 11.

‡ For each of the three sets of experiments the mice in all groups were as closely matched as possible for age (within the range of 4-6 wk at the time of treatment).

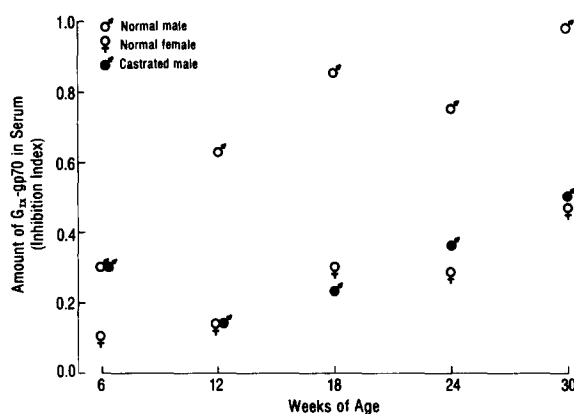


FIG. 1. Levels of G_{IX} -gp70 in the serum of normal male and female 129 mice, and of 129 males castrated at 6 wk of age (bilateral excision of testis, epididymis, and vas deferens). Each point is the mean of assays of at least five mice of the age and sex indicated.

(data not shown) indicate that the serum of young adult 129 males contains roughly four times as much G_{IX} -gp70 as the serum of age-matched 129 females.

Castration. Bilateral excision of testis, epididymis, and vas deferens reduced the s- G_{IX} -gp70 level of males to that of females (Fig. 1 and Table I; groups 4, 6, and 13). Neither unilateral excision of these three structures (group 5) nor bilateral excision of epididymis and vas deferens (group 15) affected s- G_{IX} -gp70 levels, but bilateral excision of testis alone (group 14) reduced the s- G_{IX} -gp70 level to that of normal females (group 16). Thus the testis itself is directly or indirectly responsible for the high level of s- G_{IX} -gp70 in males.

Effect of Testosterone. To determine whether the testis is the site of synthesis or secretion of s- G_{IX} -gp70, or whether the testis affects s- G_{IX} -gp70 levels by hormonal effects on organs or tissues that produce s- G_{IX} -gp70 elsewhere, we tested the effect of administering testosterone to (a) female 129 mice, and (b) male 129 mice whose s- G_{IX} -gp70 had been reduced to the female level by excision of the testes, with or without removal of epididymides and vasa deferentia. Testosterone propionate (1 mg in 0.1 ml sesame oil; Sigma Chemical Company, St. Louis, Mo.) was injected interperitoneally daily for 10 days, and s- G_{IX} -gp70 levels were measured on the next day. Testosterone restored the s- G_{IX} -gp70 levels of the castrated males to the level of normal males (Table I; group 13, control group 12). Thus the entire excess of s- G_{IX} -gp70 in males (roughly four times that of females in early adult life) can be attributed to testosterone. Furthermore, the epididymis and vas deferens are not target-organs for testosterone-mediated s- G_{IX} -gp70 production, because the s- G_{IX} -gp70 response to testosterone was not affected by presence or absence of these structures (groups 13 and 14). The content of s- G_{IX} -gp70 in female serum was more than doubled by testosterone, but did not reach the level of normal males (groups 12 and 16).

Clearance of s- G_{IX} -gp70. The higher level of s- G_{IX} -gp70 in males could possibly be due to slower clearance rather than greater production of G_{IX} -gp70, but the following tests made this unlikely: strain 129- G_{IX}^- mice each received 2 ml of a pool of serum from female 129 mice (intravenously, in divided doses, during a period of 30 min.). The recipients were (a) normal adult females, (b) females given the 10-day course of testosterone, and (c) normal adult males. Measurements of s- G_{IX} -gp70, made 3, 6, and

24 h later, showed no significant differences between the three groups, implying that clearance of s-G_{IX}-gp70 is not significantly different in males and females, and that clearance of s-G_{IX}-gp70 is not obviously affected by testosterone.

Discussion

The special interest of the gp70 family of molecules, including G_{IX}-gp70, lies in their dual role as constituents of C-type viral envelopes and also as cellular constituents inherited in Mendelian fashion independently of virus production. It is not yet known what role G_{IX}-gp70 may play in the physiology or pathology of virus-negative G_{IX}⁺ mice, although its expression in mice of certain genotypes leads to autoimmune disease (8).

gp70 occurs in the serum of various mouse strains (5, 9–12), but its presence in serum has not been traced to production by any particular tissue or organ. The 129 mouse and its congenic 129-G_{IX}⁻ partner strain (6) are particularly suitable for such a study: in 129 mice, G_{IX}-gp70 is found in the plasma membrane of thymocytes (2), and it is produced by various secretory epithelia (5). In 129 males, G_{IX}-gp70 is abundant in cells lining the epididymis; its presence in seminal fluid (5) may account for the presence of G_{IX}-gp70 (by adsorption) on sperm. At all these sites where G_{IX}-gp70 is demonstrable serologically in 129 mice, its expression is evidently governed by a single locus (*Gv-1*) because it is nowhere demonstrable in 129-G_{IX}⁻ congenic mice.

Our data exclude several possible sources of G_{IX}-gp70 in 129 serum. The level of G_{IX}-gp70 in serum was not lowered in lethally irradiated 129 mice restored with 129-G_{IX}⁻ hematopoietic cells, nor did G_{IX}-gp70 appear in the serum of lethally irradiated 129-G_{IX}⁻ mice restored with 129 cells. Therefore hematopoietic tissues and cells are not a significant source of s-G_{IX}-gp70 in either males or females. Neither is the thymus or spleen responsible, because s-G_{IX}-gp70 levels were not affected by thymectomy or splenectomy in either sex; (furthermore, in another study we have found G_{IX}-gp70 in the serum of HSFS/N mice, whose thymocytes are G_{IX}-negative).

The serum of young adult 129 males contains about four times as much G_{IX}-gp70 as does female 129 serum. For the following reasons, this difference can be attributed to testosterone: the epididymis and seminal fluid are rich in G_{IX}-gp70, and castration reduces the s-G_{IX}-gp70 level of males to that of females. But testosterone fully restored the s-G_{IX}-gp70 level of castrated males. Thus the higher level of s-G_{IX}-gp70 in males is not due to the G_{IX}-gp70 which is produced abundantly in the male reproductive tract, but to the action of testosterone on an organ or organs not yet identified.

The s-G_{IX}-gp70 level of females was more than doubled by testosterone, signifying that testosterone raises s-G_{IX}-gp70 production by an organ or organs shared by males and females. But the s-G_{IX}-gp70 level of testosterone-treated females did not reach the level of normal males or of testosterone-treated castrated males. Although this might indicate an action of testosterone on G_{IX}-gp70 produced by accessory male organs remaining after castration, it seems more likely that the higher response of castrated males, as compared with normal females, is due to imprinting of the male by testosterone in early life (13–15) or antagonism by estrogen.

The question whether testosterone acts directly on the tissue or organ supplying s-G_{IX}-gp70, or indirectly via the pituitary, or in both ways, remains to be decided by experiments now in hand. In either event the results of this study indicate production of s-G_{IX}-gp70 by an organ or tissue that has not yet been serologically identified as

G_{IX}-positive. The liver, as the source of several proteins whose concentration in serum is markedly dependent on sex (16), would from that viewpoint seem a likely source of s-G_{IX}-gp70, but there has so far been no serological evidence of G_{IX}-gp70 in this organ.

Summary

The gp70 family of glycoproteins is distinguished by the role of these molecules as constituents of C-type viral envelopes and also as Mendelian cellular constituents expressed independently of virus production.

The source of G_{IX}-gp70 in the serum of 129 strain mice, which are not overt producers of virus, could not be traced to any organ or tissue that is known to be G_{IX}-positive by serological tests. Hematopoietic tissues were excluded as source of serum G_{IX}-gp70 by tests with reciprocal radiation chimeras made from 129 and 129-G_{IX}⁻ donors and recipients. Thymus and spleen were excluded because excision of these organs did not affect levels of G_{IX}-gp70 in the serum.

The serum of young adult 129 males contains roughly four times as much G_{IX}-gp70 as adult 129 females and the levels rise in both sexes with increasing age. Castration of 129 males reduced the level of serum G_{IX}-gp70 to that of females, and the level was fully restored by testosterone. Thus the epididymis and seminal fluid, though rich in G_{IX}-gp70, do not contribute significant amounts of G_{IX}-gp70 to the serum.

The level of G_{IX}-gp70 in the serum of testosterone-treated females, though more than double that of untreated females, did not reach the level of normal males, under the conditions tested. This may signify that G_{IX}-gp70 production by males is subject to imprinting by testosterone in early life.

Evidently the main source of serum G_{IX}-gp70 is a tissue or organ that is common to males and females, is directly or indirectly responsive to testosterone, and has not so far been identified serologically as G_{IX}-positive.

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