IDENTIFICATION OF RETROVIRAL gp70 AND ANTI-gp70 ANTIBODIES INVOLVED IN CIRCULATING IMMUNE COMPLEXES IN NZB × NZW MICE*

By SHOZO IZUI, JOHN H. ELDER, PATRICIA J. McCONAHEY, AND FRANK J. DIXON

From The Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

The major envelope glycoprotein, gp70, of endogenous retrovirus found in murine strains that are susceptible to spontaneous systemic lupus erythematosus (SLE)[1] seems to play an important role in their disease. The demonstration of retroviral gp70 in circulating blood and in deposits along with host immunoglobulins (Ig) and complement in diseased glomeruli of mice with SLE supports the pathogenic role of gp70-anti-gp70 immune complexes (IC) (1–4). Indeed, four strains of mice that develop SLE all have these gp70 in circulating blood, whereas immunologically normal strains do not (5). The appearance and amounts of these IC closely parallel the course of disease in each SLE-prone mouse (5, 6).

It is clear that SLE-prone mice respond immunologically to their own retroviral gp70 by forming antibody, which subsequently becomes part of the IC found in their serum (5). Because multiple immunologically related gp70 are expressed in every mouse (7), and antibodies to one or more of them may be made (5), we have undertaken an analysis of the nature of gp70 and anti-gp70 antibodies in the circulating IC of SLE-prone mice. When we compared the gp70 isolated from circulating IC (IC-gp70) of (NZB × NZW)F_1 hybrid (NZB × W) mice with that found free in serum and that from several different retroviruses, the IC-gp70 resembled NZB xenotropic viral gp70 (NZB-X1 gp70) (8). This form of gp70 accounts for most if not all of the circulating gp70 in all strains of mice (7). In exhaustive tests for specificity, anti-gp70 antibodies isolated from circulating IC (IC-anti-gp70) preferentially bound NZB-X1 gp70 over other types of retroviral gp70.

Materials and Methods

Mice. NZB and NZW mice, originally obtained from the Laboratory Animal Center, Medical Research Council, Surrey, England, have been bred at Scripps Clinic and Research

* Supported in part by grants AI-07007, N01-CP-71018, R01-CA-25533, CA-255803, and CA-16600 from the U. S. Public Health Service, and the Cecil H. and Ida M. Green Research Endowment. Publication 2184 from the Immunology Departments, Scripps Clinic and Research Foundation, La Jolla, Calif.

[1] *Abbreviations used in this paper:* Con A, concanavalin A; FeLV, feline leukemia virus; IC, immune complex; IC-anti-gp70, anti-gp70 antibodies isolated from the circulating IC; IC-gp70, retroviral gp70 isolated from the circulating IC; MuLV, murine leukemia virus, NZB-X1, xenotropic virus predominantly expressed in NZB mice; NZB-X2, second type of xenotropic virus induced by 5-iododeoxyuridine in NZB fibroblasts in vitro; NZB×W, (NZB × NZW)F_1 hybrid; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; Staph A, *Staphylococcus aureus* that contained protein A.

Foundation, La Jolla, Calif. since 1965. Mating NZB females with NZW males in our animal colony yielded the F_1 hybrids NZB×W. BXSB and MRL/Mp (*lpr/lpr*) (designated MRL/1) mice were originally obtained from The Jackson Laboratory, Bar Harbor, Maine, and have been bred at the Scripps Clinic and Research Foundation for the past 5 yr. DBA/2, LG/J, and AKR mice were purchased from The Jackson Laboratory. Blood samples were collected from these animals by orbital sinus puncture, and the sera were stored at −20°C until use.

Retroviruses and Retroviral Envelope Glycoprotein gp70. Rauscher murine leukemia virus (MuLV), BALB/c xenotropic virus, and AKR ecotropic virus were obtained from the National Cancer Institute, Bethesda, Md. NZB xenotropic virus (NZB-X1; NZB clone 35), AKR xenotropic virus, AKR recombinant virus, and wild mouse amphotropic virus (4070A) were kindly provided by Dr. F. C. Jensen (Scripps Clinic and Research Foundation). NIH Swiss xenotropic virus (RD-ATS 124) was obtained by inoculating a human rhabdomyosarcoma cell line into antithymocyte-treated NIH Swiss mice (9). gp70 of Rauscher MuLV was purified according to the procedure of Strand and August (10). gp70 from NZB-X1, murine sera, or partially purified gp70 IC were purified by immunoaffinity column chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (7). gp70 from AKR ecotropic virus was a gift from Dr. A. F. Esser (Scripps Clinic and Research Foundation).

Antisera. Goat anti-feline leukemia virus (FeLV) antisera and goat anti-AKR ecotropic virus antisera were obtained from the National Cancer Institute. Anti-Rauscher MuLV gp70 antibodies were raised in goats by repeated injections of purified Rauscher MuLV gp70 in complete Freund's adjuvant. Anti-murine IgG came from rabbits immunized by repeated injections of murine IgG (Miles Laboratories, Inc., Elkhart, Ind.).

Radiolabeling Procedures. gp70 isolated from Rauscher MuLV, AKR ecotropic virus, NZB-X1 and NZB×W mouse serum were labeled with radioactive iodine (^{125}I) by the chloramine T method (11).

Radioimmunoassay for gp70. The concentrations of gp70 in serum samples, gradient fractions, or retrovirus preparations were determined by inhibiting the binding of goat anti-FeLV antibody to ^{125}I-labeled gp70 from Rauscher MuLV. The details of this assay were described previously (5). A standard inhibition curve was established with the buffer that contained known amounts of gp70 from sera of 2-mo-old NZB mice, and the concentration of gp70 present in tested samples was estimated by reference to the standard curve.

In some experiments, the concentrations of gp70 in sera or retroviruses were determined by a similar radioimmunoassay with ^{125}I-labeled AKR ecotropic viral gp70 and goat anti-AKR ecotropic virus antibodies. gp70 of AKR ecotropic virus was used as reference antigen for the latter radioimmunoassay.

Isolation of Free gp70 from Sera. Pooled sera from 7–10-mo-old NZB×W female mice containing free and Ig-complexed gp70 and 2-mo-old DBA/2 or AKR mice containing only free gp70 were applied to a cyanogen bromide-activated Sepharose 4B column coated with concanavalin A (Con A) (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.). Bound gp70 was eluted by 0.1 M α-methyl-D-mannoside in 0.01 M phosphate-buffered saline, pH 7.4 (PBS). To separate free gp70 from the gp70 complexed with antibodies in the materials eluted from Con A columns, the eluted materials were applied to a Sepharose 4B column that contained *Staphylococcus aureus* protein A, which selectively binds Ig-complexed gp70 (5). Unbound materials were concentrated and dialyzed against PBS, then applied to a 5–20% (wt: vol) linear sucrose density gradient in PBS and centrifuged at 36,000 rpm for 15 h at 4°C with a SW60 rotor in a Beckman L-75 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The gradients were divided into 24 fractions. The presence of gp70 in each fraction was detected by a radioimmunoassay, and IgG was detected by radial immunodiffusion in agar with rabbit anti-murine IgG antisera. Materials not bound to the protein A column contained no detectable amounts of rapidly sedimenting Ig-complexed gp70, but did have free gp70 sedimenting in the 5S fractions. gp70-rich fractions were reapplied to the sucrose density gradients, and gradient fractions containing gp70 but no detectable amounts of IgG were used as free gp70 in further experiments.

Isolation of IC-gp70 and IC-Anti-gp70. Circulating gp70 IC were obtained from pooled sera of 7–10-mo-old NZB×W female mice by affinity column chromatography. Serum gp70, free and complexed, were first isolated from the Con A column, as described in the above section, then

applied to a protein A column. The bound gp70 IC were then eluted with 1 M potassium thiocyanate (KSCN) in PBS. Eluates thus obtained were immediately concentrated and applied to a 5–20% sucrose density gradient in PBS containing 1 M KSCN as described previously (5). The resulting 24 fractions were immediately dialyzed against PBS, and the gp70 and IgG contents were determined. To obtain relatively pure IgG anti-gp70 antibodies or gp70, the enriched fractions of each were passed several times through KSCN-gradients. Gradient fractions containing IgG but not detectable amounts of gp70 were used as IgG anti-gp70 antibodies from the IC (IC-anti-gp70) and those containing gp70 but no measurable amounts of IgG were used as gp70 from the IC (IC-gp70).

Two-Dimensional Peptide Fingerprinting. Tryptic peptide fingerprints were performed on immune-precipitated bands from SDS-PAGE gels as previously described (7, 12).

Binding of IC-Anti-gp70 to gp70 from Various Sources. Sera or retrovirus preparations containing constant amounts of gp70 were incubated with various amounts of IC-anti-gp70 isolated from NZB×W mice or goat anti-AKR ecotropic virus antisera at 4°C overnight. To determine the amounts of gp70 bound to IC-anti-gp70 or goat anti-AKR ecotropic virus antisera, the mixtures were depleted of IgG by adsorption with 0.025 ml of packed *S. aureus* that contained protein A (Staph A; provided by Dr. M. J. Buchmeier, Scripps Clinic and Research Foundation) for 30 min at room temperature. After the removal of IgG-bound Staph A by centrifugation, the amounts of gp70 in the supernate were determined by radioimmunoassay. As a control, serum or retroviral gp70 were incubated with normal mouse IgG or normal goat serum and then treated with Staph A. From the concentrations of gp70 in both supernates, the amounts of gp70 bound by IC-anti-gp70 or goat antisera were calculated. It should be noted that, in the absence of anti-gp70 antibodies, <5% of serum gp70 or viral gp70 adsorbed to the Staph A. In some experiments, the mixtures were depleted of IgG by immune precipitation with rabbit anti-murine IgG antisera, from which anti-goat IgG activity was preabsorbed by goat IgG-coated immunoadsorbent columns, because anti-goat IgG activity interferes with the radioimmunoassay for gp70. As a control, the mixtures were treated with normal rabbit serum.

Results

Characterization of IC-gp70 from NZB× W Mice. Approximately 30% of circulating gp70 was complexed with Ig when we processed a serum pool obtained from 7–10-mo-old NZB×W female mice by quantitative absorption with Staph A. Free gp70 as well as gp70 complexed with anti-gp70 antibodies in this pooled serum sample were then isolated by affinity column chromatography and sucrose density gradient ultracentrifugation.

The isolated IC-gp70 was first characterized immunochemically by two competitive radioimmunoassays, one with anti-FeLV antibody and ^{125}I-Rauscher MuLV gp70 and the other with anti-AKR ecotropic virus antibody and ^{125}I-AKR ecotropic viral gp70. Relative inhibitory activities of IC-gp70 in both radioimmunoassays were then compared with those of free gp70 in sera of NZB×W, DBA/2, or AKR mice and with those of various retroviruses. IC-gp70 efficiently inhibited the reactivity of anti-FeLV antibodies to ^{125}I-Rauscher MuLV gp70, but only very slightly inhibited the binding of anti-AKR ecotropic virus antibodies to ^{125}I-AKR ecotropic viral gp70 (Fig. 1).

Inhibition of IC-gp70 in two radioimmunoassays resembled that by sera from NZB×W and DBA/2 mice that predominantly contain NZB-X1 gp70 and that by xenotropic viruses from NZB and AKR mice and amphotropic virus from wild mice. In contrast, the inhibitory patterns by serum gp70 from AKR mice and by gp70 from both AKR recombinant virus and AKR ecotropic virus differed from that by IC-gp70. AKR serum that expresses AKR ecotropic viral gp70 as well as NZB-X1 gp70, and AKR recombinant virus containing both xenotropic and ecotropic genomes, not only competed in the anti-FeLV-^{125}I-Rauscher MuLV gp70, but also inhibited,

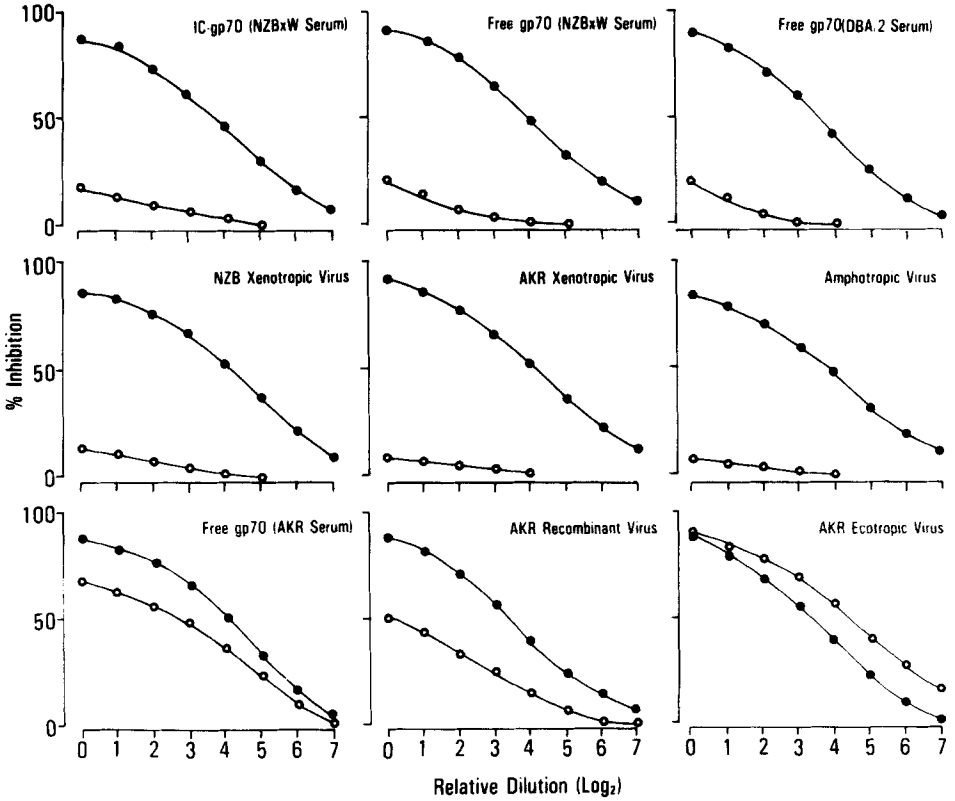

FIG. 1. Inhibitory pattern of competitive binding by IC-gp70, serum-free gp70, and various retroviral gp70 in two radioimmunoassays: with anti-FeLV antibody and ^{125}I-Rauscher MuLV gp70 (●) and with anti-AKR ecotropic virus antibody and ^{125}I-AKR ecotropic viral gp70 (○). Various concentrations of gp70 preparations were serially diluted in buffer. Each point represents the percent inhibition of binding of ^{125}I-gp70 to antibodies in both assays at each dilution of samples.

though to a lesser extent, the binding of ^{125}I-AKR ecotropic gp70 to anti-AKR ecotropic virus antibodies. AKR ecotropic virus exhibited stronger inhibitory activity in the assay with anti-AKR ecotropic virus antibodies and ^{125}I-AKR ecotropic viral gp70 than in the other assay.

Because immunochemical analysis could not distinguish whether IC-gp70 was of xenotropic origin or amphotropic origin, the molecular nature of IC-gp70 was investigated by tryptic peptide mapping analysis. Shown in Fig. 2 are the tryptic fingerprints of gp70 from the predominant xenotropic virus endogenous to NZB mice (NZB-X1) (8) (Fig. 2A), free gp70 from the serum of NZB×W mice (Fig. 2B), and IC-gp70 of NZB×W mice (Fig. 2C). As reported previously (7, 8), the predominant free gp70 of serum in NZB×W mice was identical to NZB-X1 gp70 and possessed a rapidly migrating peptide (Fig. 2, arrow) that was lacking from other gp70 including AKR ecotropic virus, amphotropic virus, and xenotropic viruses isolated from murine strains other than NZB and NZB×W. However, if the fingerprint of free gp70 isolated from serum was overexposed, markers of a second, closely related gp70 were evident (Fig. 2, brackets). These marker peptides were reminiscent of, but not identical to, the pattern from the same region reported previously for other xenotropic viruses (8).

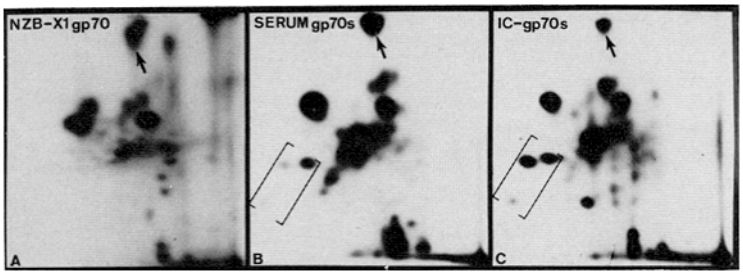

FIG. 2. Tryptic peptide fingerprints of gp70 from sera of NZB×W mice and from NZB xenotropic virus. gp70 were isolated by immunoaffinity chromatography and radiolabeled before analysis. (A) NZB-X1 gp70 from the predominant xenotropic virus of NZB mice (8); (B) free gp70 in sera from 7–10-mo-old NZB×W mice; (C) IC-gp70 from the circulations of 7–10-mo-old NZB×W mice. Arrows indicate a marker peptide characteristic of NZB-X1 gp70. Brackets in B and C indicate constellation of peptides that denotes a second closely related gp70 present in serum. Note that both marker peptides are present in IC-gp70 and serum-free gp70 as well.

Both the NZB-X1 marker (arrow) and markers of the second gp70 (brackets) were present in IC-gp70, indicating that IC-gp70 were structurally identical to the free form of serum gp70. Moreover, the tryptic fingerprints of IC-gp70 were different from those of other types of non-NZB xenotropic viruses, amphotropic virus, as well as ecotropic virus (7, 8).

Characterization of IC-Anti-gp70 Antibodies. The binding activity of IC-anti-gp70 to serum gp70 from various strains of mice or to viral gp70 of various retrovirus preparations was determined by using Staph A to precipitate gp70 bound by IC-anti-gp70. IC-anti-gp70 reacted almost equally well with free gp70 from sera of 2-mo-old mice from SLE-prone strains (NZB, NZB×W, MRL/1 and male BXSB) and from normal strains (NZW, LG/J, DBA/2, and female BXSB) (Table I). When binding of IC-anti-gp70 to various viral gp70 was compared with that to serum gp70, only gp70 from NZB-X1 functioned equivalently to serum gp70 (Table I). Binding was moderate when gp70 from other types of non-NZB xenotropic viruses (BALB/c, AKR, or NIH Swiss mice) were tested. However, IC-anti-gp70 only minimally bound gp70 of AKR ecotropic virus, AKR recombinant virus, or Rauscher MuLV. There was essentially no binding to amphotropic viral gp70. Similar results were obtained when the binding activity was determined by the precipitation of Ig-bound gp70 with anti-murine IgG antisera.

Because the radioimmunoassay with anti-FeLV antibodies and ^{125}I-Rauscher MuLV gp70 is less efficient in detecting AKR ecotropic viral gp70 than that with anti-AKR ecotropic virus antibodies and ^{125}I-AKR ecotropic viral gp70, the binding activity of IC-anti-gp70 to AKR ecotropic viral gp70 was determined by the latter radioimmunoassay. However, even then, $\leq$10% binding resulted (Table II).

We next used a more quantitative method to compare the binding of IC-anti-gp70 to several gp70. For this purpose, constant amounts of gp70 from NZB×W mouse sera or from retroviruses were incubated with increasing amounts of IC-anti-gp70, and the percent binding by IC-anti-gp70 was determined. At all concentrations tested, the binding capacities of IC-anti-gp70 to NZB×W serum gp70 and to NZB-X1 gp70 were essentially identical (Fig. 3) and contrasted markedly to the much lower binding of AKR ecotropic viral gp70. At the concentration of IC-anti-gp70 that bound ~50% of serum gp70 or NZB-X1 gp70, only 10% of AKR ecotropic viral gp70 was specifically

TABLE I

Binding of IC-Anti-gp70 to gp70 from Various Sources

Source of gp70	Binding of gp70 in experiment*		
	1	2	3
		%	
Serum‡			
NZB×W♀	66.5§	58.4	54.1
NZB♀	64.1	NT‖	NT
MRL/1♀	63.1	NT	NT
BXSB♂	61.9	NT	NT
BXSB♀	57.5	NT	NT
NZW♀	50.8	NT	NT
LG/J♂	62.9	NT	NT
DBA/2♂	66.8	NT	NT
Retrovirus			
NZB-X1	59.5	52.6	41.8
BALB/c xenotrope	24.9	NT	NT
AKR xenotrope	25.4	NT	NT
NIH Swiss xenotrope	27.6	NT	NT
AKR ecotrope	10.7	10.2	9.0
AKR recombinant	11.3	13.1	NT
Rauscher MuLV	6.2	0.0	6.5
Amphotrope	0.0	0.0	2.4

* Binding of gp70 by IC-anti-gp70 was determined by quantitating the amounts of gp70 absorbed specifically with Staph A in the presence of IC-anti-gp70. Without IC-anti-gp70, Staph A absorbed <5% of serum or retroviral gp70.

‡ Pooled from 10 2-mo-old mice.

§ Mean of triplicates.

‖ Not tested.

precipitated. Because 10 times less IC-anti-gp70 bound ~10% of NZB-X1 gp70, the binding affinity of IC-anti-gp70 to NZB-X1 gp70 was ~10 times greater than that to AKR ecotropic viral gp70.

As further confirmation of this high affinity, we investigated whether IC-anti-gp70 preferentially react with NZB-X1 gp70 when offered a mixture containing AKR ecotropic viral gp70 as well. For this purpose, IC-anti-gp70 was incubated with AKR mouse serum that contains both NZB-X1 gp70 and AKR ecotropic viral gp70. To differentiate the binding of these gp70, we used two radioimmunoassays: the one with anti-FeLV antisera and ^{125}I-Rauscher MuLV gp70 detects the binding of NZB-X1 gp70 and to a lesser degree AKR ecotropic viral gp70; the second radioimmunoassay with anti-AKR ecotropic virus antibodies and ^{125}I-AKR ecotropic viral gp70 detects only AKR ecotropic viral gp70 efficiently. According to the former radioimmunoassay, IC-anti-gp70 bound a significant amount of gp70 from AKR serum, whereas the latter radioimmunoassay showed essentially no binding (Table II). Similarly, when IC-anti-gp70 was incubated with the mixture of NZB-X1 virus and AKR ecotropic virus, significant binding was observed only by using the former radioimmunoassay

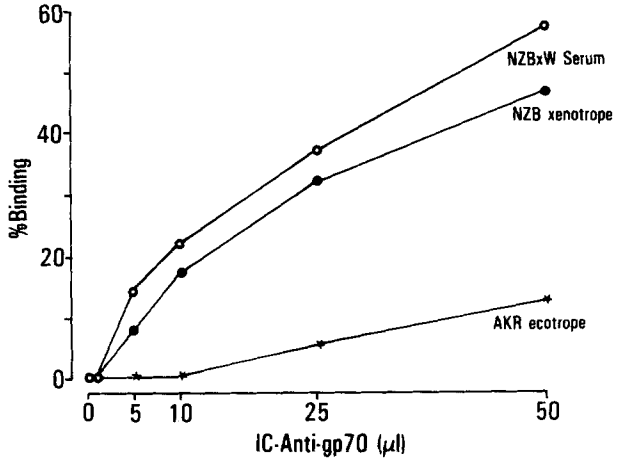

FIG. 3. Binding of IC-anti-gp70 to serum-free gp70 from sera of 2-mo-old NZB×W mice (○), NZB-X1 gp70 (●), and AKR ecotropic viral gp70 (★). Constant amounts of gp70 were incubated with increasing amounts of IC-anti-gp70 at 4°C overnight and the percent binding of these gp70 by IC-anti-gp70 was determined by using Staph A to precipitate gp70 bound to IC-anti-gp70. Each point represents the mean of triplicates.

TABLE II

Binding of IC-Anti-gp70 to NZB Xenotropic Viral and AKR Ecotropic Viral gp70

Antibodies	Source of gp70	Anti-FeLV* ^{125}I-Rgp70	Anti-AKR virus‡ ^{125}I-AKR gp70
		%	
IC-anti-gp70	AKR serum‖	62.2¶	0.0
IC-anti-gp70	NZB xenotropic virus	46.4	0.0
IC-anti-gp70	AKR ecotropic virus	9.6	8.2
IC-anti-gp70	NZB xenotropic virus + AKR ecotropic virus	29.7	0.0
Anti-AKR virus§	AKR serum	55.1	66.4

* Radioimmunoassay with goat anti-FeLV antibody and ^{125}I-Rauscher MuLV gp70 (^{125}I-Rgp70) that recognizes both NZB xenotropic and AKR ecotropic viral gp70 efficiently.

‡ Radioimmunoassay with goat anti-AKR ecotropic virus antibody and ^{125}I-AKR ecotropic viral gp70 that recognizes only AKR ecotropic viral gp70 efficiently.

§ Obtained from goats immunized with AKR ecotropic virus.

‖ Containing both NZB xenotropic viral and AKR ecotropic viral gp70.

¶ Mean of triplicates.

that detects NZB-X1 gp70. This result clearly indicates that IC-anti-gp70 preferentially recognized NZB-X1 gp70 over AKR ecotropic viral gp70. It should be noted that the serum from goats immunized with AKR ecotropic virus bound AKR serum gp70 in both radioimmunoassays.

Discussion

Our results concern the nature of IC-gp70 in the several strains of mice that are genetically predisposed to murine SLE, produce antibodies against their own serum

gp70, and consequently form IC (5, 6). We have now demonstrated that these IC-gp70 have the immunologic and structural characteristics of gp70 from the NZB xenotropic virus NZB-X1 expressed dominantly in sera of all SLE-prone and normal mice, and distinct from gp70 belonging to viruses with other tropisms. This conclusion is supported by several experimental observations. First, structural analysis of the IC-gp70 by tryptic peptide maps indicate that the IC-gp70 are similar to NZB-X1 gp70, but differ from gp70 of AKR ecotropic virus, amphotropic virus, and xenotropic viruses isolated from murine strains other than NZB and NZB×W mice, including NIH Swiss, AKR, NZW, C57BL/6, BALB/c, and wild mice (7, 8). Second, the IC-gp70 do not show structural relatedness either to gp70 of a second type of xenotropic virus induced by 5-iododeoxyuridine in NZB fibroblasts in vitro (NZB-X2) (8) or to gp70 expressed on the surfaces of thymocytes and spleen cells (8). Both NZB-X2 and cell surface-associated gp70 are structurally different from NZB-X1 gp70, but have molecular characteristics similar to those of xenotropic viral gp70 isolated from strains other than NZB and NZB×W mice (8). Third, the characterization of IC-gp70 determined by two different competitive radioimmunoassays that distinguish AKR ecotropic viral gp70 from xenotropic viral gp70 reveals that the IC-gp70 do not carry the determinants specific for the AKR ecotropic viral gp70, ruling out the possible involvement of AKR ecotropic virus or recombinant virus formed between ecotropic virus and xenotropic virus (12).

Tryptic peptide maps of the IC-gp70 reaffirm that the IC-gp70 are structurally identical to gp70 circulating free in sera of not only SLE-prone mice but also normal murine strains (7). It should be pointed out that this free gp70 in serum is molecularly unchanged during the course of murine SLE, excluding the possibility that affected mice produce a new or modified type of gp70 that elicits their immune responses. Because IC-gp70 carry unidentified peptides that are not components of any retroviral gp70 tested, including NZB-X1 gp70, the involvement of retroviral gp70 other than NZB-X1 gp70 is not excluded. However, it should be emphasized that these unidentified peptides are also present in freely circulating gp70 of every murine strain. Therefore, one must conclude that IC-gp70 is not a unique type, but is commonly expressed in sera of virtually all mice.

The immunologic specificity of IC-anti-gp70 antibodies was assayed in terms of binding to various retroviral gp70. The results show that IC-anti-gp70 are primarily directed to NZB-X1 gp70 as well as serum gp70, with minimal cross-reactivity to other retroviral gp70. This conclusion is drawn from the following evidence. First, IC-anti-gp70 binds NZB-X1 gp70 and serum gp70 considerably better than the other retroviral gp70 tested. Second, quantitative studies show that the affinity of IC-anti-gp70 for NZB-X1 gp70 and for serum gp70 is 10 times higher than for AKR ecotropic viral gp70. Finally, IC-anti-gp70 selectively recognize NZB-X1 gp70 when incubated with a mixture of NZB-X1 gp70 and AKR ecotropic viral gp70. This observation excludes the possibility that anti-AKR ecotropic viral gp70 antibodies or anti-Rauscher MuLV gp70 antibodies occasionally found in sera of NZB×W and MRL/1 mice (5, 13) participate in the formation of circulating gp70-anti-gp70 IC. In fact, the presence of such antibodies in sera of NZB×W or MRL/1 mice does not correlate at all either with serum levels of gp70-anti-gp70 IC or with the development of renal disease (5). Further, these antibodies are found in several strains of normal mice such as NZW and LG/J, although two other SLE strains, NZB and BXSB mice

that develop gp70-anti-gp70 IC, do not produce detectable amounts of these antibodies (5, 14).

It is noteworthy that IC-anti-gp70 react equally well with free gp70 in sera of SLE strains of mice and in all normal strains of mice tested. This confirms that sera from all these mice express the same type of gp70, which is similar to NZB-X1 gp70, although AKR mice express an additional AKR ecotropic viral gp70 in their sera. Furthermore, the antibody involved in this IC formation is not directed to a type of gp70 expressed only in the SLE-prone mice, but rather is directed to a ubiquitous molecule of all the mice.

Our observations that the IC-gp70 are identical to free gp70 in sera of all murine strains and that the IC-anti-gp70 reacts preferentially with NZB-X1 gp70 as well as serum gp70 suggest that the abnormality of SLE mice manifested as production of antibodies to their own retroviral serum gp70 and formation of IC is not the result of an unusual kind of gp70, but rather of the unique ability of these mice to make antibody to their own NZB-X1 gp70. Such an abnormal immune response may result from their well-known immunologic dysfunction, for example, lack of T cell regulatory function (15, 16), loss of suppressor T cells (17, 18), and/or increased B cell activity (19, 20). Therefore, like responses to many other autoantigens, the anti-gp70 response of mice with SLE may reflect the abundant formation of Ig reactive with multiple available antigens, among them NZB-X1 gp70, by hyperactive B cells.

Summary

Retroviral gp70 and anti-gp70 antibodies were isolated from circulating immune complexes (IC) of 7–10-mo-old (NZB × NZW)F_1 mice, after which the nature and origin of this gp70 (IC-gp70) and the immunologic characteristics of these anti-gp70 antibodies (IC-anti-gp70) were investigated. Immunochemical and structural analyses of IC-gp70 demonstrated that among multiple immunologically related gp70 expressed in all mice, the IC-gp70 had characteristics similar to those of NZB xenotropic viral gp70 (NZB-X1 gp70) that is commonly present in sera of virtually all strains of mice. The study of binding by IC-anti-gp70 antibodies to retroviral gp70 from various sources showed that the IC-anti-gp70 were primarily directed to NZB-X1 gp70 as well as serum gp70. These data strongly suggest that the abnormality of murine strains with systemic lupus erythematosus causing them to produce antibodies to their own xenotropic viral gp70 and to form IC with serum gp70 is not based on their expression of an unusual type of gp70, but rather their ability to make an antibody to NZB-X1 gp70, probably as a result of their immunologic dysfunction.

We thank Ms. Paula Eisenhart and Mr. John Clark for expert technical assistance, and Ms. Phyllis Minick for excellent editorial assistance.

Received for publication 4 February 1981.

References

1. Yoshiki, T., R. C. Mellors, M. Strand, and J. T. August. 1974. The viral envelope glycoprotein of murine leukemia virus and pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J. Exp. Med.* **140:**1011.

2. Strand, M., and J. T. August. 1976. Oncornavirus envelope glycoprotein in serum of mice. *Virology.* **75:**130.
3. Lerner, R. A., C. B. Wilson, B. C. Del Villano, P. J. McConahey, and F. J. Dixon. 1976. Endogenous oncornaviral gene expression in adult and fetal mice: quantitative, histologic and physiologic studies of the major viral glycoprotein, gp70. *J. Exp. Med.* **143:**151.
4. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* **148:**1198.
5. Izui, S., P. J. McConahey, A. N. Theofilopoulos, and F. J. Dixon. 1979. Association of circulating retroviral gp70-anti-gp70 immune complexes with murine systemic lupus erythematosus. *J. Exp. Med.* **149:**1099.
6. Izui, S., V. E. Kelley, P. J. McConahey, and F. J. Dixon. 1980. Selective suppression of retroviral gp70-anti-gp70 immune complex formation by prostaglandin E_1 in murine systemic lupus erythematosus. *J. Exp. Med.* **152:**1645.
7. Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner. 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differential antigens encoded by a multi-gene family. *Nature (Lond.).* **267:**23.
8. Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, T. M. Chused, H. C. Morse, J. W. Hartley, and W. P. Rowe. 1980. Differential expression of two distinct xenotropic viruses in NZB mice. *Clin. Immunol. Immunopathol.* **15:**493.
9. Todaro, G. J., P. Arnstein, W. P. Parks, E. H. Zennette, and R. J. Huebner.1973. A type-C virus in human rhabdomyosarcoma cells after inoculation into NIH Swiss mice treated with antithymocyte serum. *Proc. Natl. Acad. Sci. U. S. A.* **70:**859.
10. Strand, M., and J. T. August. 1973. Structural proteins of ribonucleic acid viruses. Interspec II, a new interspecies antigen. *J. Biol. Chem.* **248:**5627.
11. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* **29:**185.
12. Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Harlety, and W. P. Rowe. 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants. *Proc. Natl. Acad. Sci. U. S. A.* **74:**4676.
13. Aoki, T., E. A. Boyse, L. J. Old, E. de Harven, U. Hämmerling, and H. A. Wood. 1970. G (Gross) and H-2 cell-surface antigens: location on Gross leukemia cells by electron microscopy with visually labeled antibody. *Proc. Natl. Acad. Sci. U. S. A.* **65:**569.
14. Ihle, J. N., and M. G. Hanna, Jr. 1977. Natural immunity to endogenous oncornaviruses in mice. *Contemp. Top. Immunobiol.* **6:**169.
15. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T-cell sets. II. Physiologic role of feedback inhibition in vivo: absence in NZB mice. *J. Exp. Med.* **147:**1116.
16. Cantor, H., and R. K. Gershon. 1979. Immunologic circuits: cellular composition. *Fed. Proc.* **38:**2058.
17. Barthold, D. R., S. Kysela, and A. D. Steinberg. 1974. Decline in suppressor T cell function with age in female NZB mice. *J. Immunol.* **112:**9.
18. Krakauer, R. S., T. A. Waldmann, and W. Strober. 1976. Loss of suppressor T cells in adult NZB/NZW mice. *J. Exp. Med.* **144:**662.
19. Moutsopoulos, H. M., M. Boehm-Truitt, S. S. Kassan, and T. M. Chused. 1977. Demonstration of activation of B lymphocytes in New Zealand black mice at birth by an immunoradiometric assay for murine IgM. *J. Immunol.* **119:**1639.
20. Izui, S., P. J. McConahey, and F. J. Dixon. 1978. Increased spontaneous polyclonal activation of B lymphocytes in mice with spontaneous autoimmune disease. *J. Immunol.* **121:**2213.