THE VIRAL ENVELOPE GLYCOPROTEIN OF MURINE LEUKEMIA VIRUS AND THE PATHOGENESIS OF IMMUNE COMPLEX GLOMERULONEPHRITIS OF NEW ZEALAND MICE*

By TAKASHI YOSHIKI, ROBERT C. MELLORS, METTE STRAND, AND J. T. AUGUST

(From The Hospital for Special Surgery, The New York Hospital-Cornell University Medical College, the Department of Pathology, Cornell University Medical College, New York 10021, and the Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461)

The New Zealand black (NZB) mouse strain and its F_1 hybrid with the New Zealand white (NZW) strain, B/WF_1, are a widely studied model of human diseases of unknown etiology. These include idiopathic glomerulonephritis, systemic lupus erythematosus, related connective tissue and autoimmune diseases, and malignant lymphoma (see reviews 1–3).

Several studies have implicated murine leukemia virus (MuLV)[1] in the pathogenesis of the spontaneous diseases of New Zealand mice (4–8). MuLV-related antigens appear early in the life of NZB mice, and antibodies to the endogenous virus in later life, accompanied by the manifestation of immune complex glomerulonephritis (5). These antibodies were found by immunoelectron microscopy to react with viral envelope components (9–11). B/WF_1 hybrid mice show an earlier production of MuLV-related antigens and antibody as compared to NZB mice, correlating with the earlier onset of proteinuria and mortality from lupus-like glomerulonephritis. MuLV-related antigens and antibody have been demonstrated in the eluates of nephritic kidneys, and the antigens located in the glomerular lesions of NZB and B/WF_1 mice by the immunofluorescence method (5, 6). As the antiserum used in the previous studies contained antibodies to several MuLV-related antigens, it is not known whether one or several of the viral proteins is involved in the glomerular disease.

In the present report we describe the use of monospecific antisera for the analysis of specific viral proteins that could be of significance in the pathogenesis of glomerulonephritis in these mice. One of the proteins is the major internal structural component of MuLV

* This work, Research Department publication no. 516, was supported by U. S. Public Health Service Rsearch Grant no. CA-14928 and in part by research grants from the Susan Greenwall Foundation, Inc. and from the Virginia and D. K. Ludwig Foundation, Inc. Aspects of the study were also supported by U. S. Public Health Service contract 71-2251 within the Virus Cancer Program of the National Cancer Institute and by grant GM 11301-11 from the National Institute of General Medical Sciences.

[1] *Abbreviations used in this paper:* MuLV, murine leukemia virus.

with a mol wt of 30,000 (p30). The other is the major glycoprotein constituent of MuLV which recently was purified and found to be composed of two glycopeptides of apparent mol wt of 69,000 and 71,000 (gp69/71) (12, 13) and has been shown by virus neutralization and immunofluorescence studies with anti-MuLV gp69/71 serum to be a component of the virion envelope (reference 14 and footnote 2).

We now present evidence which shows that New Zealand mice contain an extremely high tissue and serum concentration of gp69/71 and that the virion envelope glycoprotein is selectively deposited in the glomerular lesions as compared to p30. These findings suggest that the expression of this specific viral envelope protein is related to the pathogenesis of immune complex glomerulonephritis of New Zealand mice.

Materials and Methods

Mice. NZB, NZW, and their F_1 hybrid mice, B/WF_1 and W/BF_1 were from our colonies. AKR/J and C57BL/6J mice were purchased from Jackson Laboratories, Bar Harbor, Maine. These mice were maintained under a specific pathogen-free environment.

Purification of Viral Proteins. The major structural protein of Rauscher MuLV (p30) and the membrane glycopeptides of Rauscher MuLV (gp69/71) were purified by phosphocellulose column chromatography and Sephadex gel filtration, as previously described (12).

Antisera. Goat anti-Rauscher MuLV p30 (anti-p30) and goat anti-Rauscher MuLV gp69/71 (anti-gp69/71) sera were obtained from goats immunized by repeated injections of purified proteins in Freund's adjuvant. The monospecific anti-p30 serum chiefly contains antibodies directed against the group-specific and interspecies determinants of p30 protein with a smaller fraction of type-specific antibodies (13). The anti-gp69/71 serum chiefly contains type-specific and group-specific and a smaller fraction of interspecies antibodies (13). (W/Fu × BN)F_1 anti-W/Fu(C58NT)D(anti-NTD) serum produced in (W/Fu × BN)F_1 rats bearing transplantable leukemia (C58NT)D induced in the W/Fu rat by wild-type MuLV was kindly supplied by Doctors E. Stockert and L. J. Old (Sloan-Kettering Institute for Cancer Research, New York). Antibody specificities in this serum, except natural heteroantibody, are directed to antigens related to MuLV including the structural proteins of MuLV, a viral envelope component (9–11) and the group and interspecies antigens of the p30 protein (15–19), GCSA (a) and (b) (15), and GIX (20, 21). As the immunoglobulins of these sera reacted with normal cellular components of mouse tissues, the sera were absorbed with C57BL/6J spleen and thymus cells in order to remove these reactants, as described elsewhere (22).

Radioimmunoassay. Assay of viral protein was carried out by radioimmunoassay as described by Hunter (23). The reaction mixture contained the following: 0.005 ml normal rabbit serum, 0.01 ml ^{125}I-labeled virus protein (1 or 2 ng of protein containing 10^4 to 10^5 counts/min/ng), either 0.15 ml of cell extract or 0.01 ml of purified virus or viral protein as competing proteins, and as the final addition 0.01 ml of rabbit anti-Theilen feline leukemia virus serum diluted in TEN-buffer containing 20 mg/ml of crystalline bovine serum albumin and added at a concentration which precipitated approximately 50% of the ^{125}I-labeled antigen. The competing proteins were diluted in TEN-buffer (20 mM Tris-hydrochloride, pH 7.6, 1 mM EDTA, and 100 mM NaCl) and Triton X-100 (0.2% final concentration), and contained 20 mg/ml of carrier crystalline serum albumin for purified virus or viral proteins and 2 mg/ml for cell extracts. The total vol of the assay was adjusted to 0.2 ml with TEN-buffer containing 0.2% Triton X-100 and 2 mg/ml crystalline bovine serum albumin. The reaction mixture was incubated at 37°C for 15–18 h, after which 0.04 ml of goat antirabbit IgG serum was added. Incubation was continued at 37°C for an additional 2 h and followed by overnight incubation at 2–4°C. Cold TEN-buffer (0.5 ml) was added and the precipitate was collected by

[2] Ikeda, H., T. Pincus, T. Yoshiki, M. Strand, J. T. August, E. A. Boyse, and R. C. Mellors. 1974. Biological expression of antigenic determinants of murine leukemia virus proteins gp69/71 and p30. Manuscript submitted for publication.

centrifugation at 4°C. The pellet was washed twice with 0.5 ml of TEN-buffer and the ^{125}I-labeled antigen present in the precipitate was measured in a gamma counter.

Perfusion of Mouse Kidney. Mice were anesthesized by ether and the kidneys were perfused in situ with cold Earle's balanced salt solution until macroscopically free of blood. Two or three blocks of kidney tissues were rapidly frozen at −70°C for immunofluorescence study, and the remainder was fixed with Vandegrift's fixative for light microscopic examination.

Kidney Eluate. Acid-buffer (pH 3.2) eluate after an elution step with deoxyribonuclease was prepared as previously described (6, 24).

Immunofluorescence Analysis of Kidney Section. MuLV-related antigens in the kidneys were located by treating frozen sections of perfused kidneys with either anti-p30 or anti-gp69/71 or anti-NTD sera, followed by fluoresceinated antibody to goat and rat gamma globulins, respectively. Fluoresceinated antibodies were absorbed with mouse gamma globulins in order to remove cross-reacting antibodies to mouse gamma globulins. Autologous mouse gamma globulins located in the kidneys were examined directly by treating the sections with fluoresceinated goat antimouse gamma globulin antibody that had been absorbed with mouse liver powder to remove nonspecific fluorescence. The specificity of fluorescence reaction of kidneys was tested by absorbing the antiserum with viral antigens as described previously (25).

Immunofluorescence Analysis of Fixed Cells. The preparation of acetone-fixed cells on slides and the immunofluorescence analysis of acetone-fixed cells were described elsewhere (reference 26 and footnote 2). The standard target cells were transplanted leukemia $E_{\delta}G2$ originally induced in C57BL/6J mouse by passage A Gross virus (27). The immunofluorescence absorption test was carried out as follows: 20 μl of antiserum two dilutions below the fluorescence end point and the same volume of serially diluted antigen were mixed and incubated overnight at 4°C. The residual antibody activity was tested on acetone-fixed $E_{\delta}G2$ cells by immunofluorescence test. The result of absorption was expressed as the maximal dilution of antigen capable of removing antibody activity from the given dilution of the antiserum.

Elution of Kidney Sections. Perfused kidney sections were incubated in 0.12 M glycine buffer (pH 3.0) for 45 min at 37°C with constant shaking in order to elute bound immunoglobulins from the glomeruli as described by Oldstone et al. (28).

Immunodiffusion Test. Ouchterlony plates prepared with 0.5% agarose in phosphate-buffered saline, pH 7.2, containing 0.03% sodium azide were incubated at room temperature in a humidified chamber. Optimal precipitation occurred within 24 h. The pooled serum used as antigen was concentrated to one-fifth of original volume by Minicon-B (Amicon Corp., Scientific System Div., Lexington, Mass.) at 4°C.

Histology. Hematoxylin and eosin, and periodic acid-Schiff staining were carried out as usual. The histologic grading of nephritis was previously described (29).

Urinalysis. Test for proteinuria was performed as previously described (29). 2+ positive reactions were graded as significant proteinuria, and 3+ as highly significant proteinuria.

Results

Tissue Concentration of Viral Proteins. The concentration of viral p30 and gp69/71 in extracts of spleens of NZB, NZW, B/WF_1, and W/BF_1 mice was measured by radioimmunoassay. A heterologous assay system with antifeline leukemia virus serum and murine virus ^{125}I-labeled protein as antigen was utilized so as to restrict antibody binding specifically to common interspecies antigenic determinants. This choice of a heterologous system was essential to a quantitative assay as previous experiments have shown that the concentration and antibody affinity of the interspecies antigens were indistinguishable among the several different murine viruses tested, Rauscher, Friend, Moloney and Gross, and AKR. This was in contrast to the results obtained by use of a homologous assay system of antimurine virus serum and the murine virus proteins, in which case the p30 or gp69/71 proteins of other viruses would not

compete for binding of type-specific antigens of the labeled protein (13).

Extremely high concentrations of gp69/71 were detected, this viral envelope protein being present in large excess over the viral p30 internal protein and comprising almost 0.1% of the total protein of the spleen extract (Table I). By comparison, a high incidence leukemia strain AKR contained 135 ng p30/mg spleen protein and 50 ng gp69/71/mg spleen protein, and a low incidence leukemia strain C57BL/6J contained even lower concentrations of these proteins, 17 and 22 ng/mg spleen protein, p30 and gp69/71, respectively. In other studies

TABLE I

p30 and gp69/71 in Spleens of New Zealand Mice

Strain	Age	p30	gp69/71
	mo	*ng viral protein/mg total spleen protein**	
NZB	9	205	700
	10	200	825
	11	365	875
NZW	5	66	660
	5	83	675
	6	100	665
B/WF_1	3	100	780
	3	100	660
	3	134	700
W/BF_1	2	77	675
	3	118	760
	3	100	770
AKR‡	2	135	50
C57BL/6‡	2	17	22

Radioimmunoassay was performed as described in the Materials and Methods.

* Values represent single determinations of individual spleens.

‡ Mice were obtained by the courtesy of Dr. F. Lilly (Albert Einstein College of Medicine, New York).

(30), these proteins in spleens of a large number of other mouse strains have been measured by the same assay and in no case did the concentrations of viral proteins approach those of the New Zealand mice, particularly in the case of gp69/71.

The relative concentration of viral p30 and gp69/71 in the pooled sera of NZB, NZW, B/WF_1, and W/BF_1 was measured semiquantitatively by immunofluorescence absorption test (Table II). A large amount of gp69/71 was demonstrated in the serum of NZB, NZW, and their F_1 hybrid mice at various ages. In contrast, p30 protein was not demonstrated by the assay method employed. The concentrations of the proteins were also measured by radioimmunoassay. There was a 1,000-fold greater concentration of gp69/71, 25 μg/ml, than of p30, 0.02 μg/ml, in the serum of each of these mice. The identity of these proteins was also

TABLE II

p30 and gp69/71 in the Sera of New Zealand Mice

Strain	Age	Antigen titer*	
		p30	gp69/71
	mo		
NZB	3–5	0	32
	6–12	0	32
	13–19	0	32
	>20	0	16
NZW	5–8	0	32
	10–12	0	32
	13–15	0	32
	16–24	0	32
B/WF_1	2–4	0	32
	5–7	0	16
	8–10	0	32
W/BF_1	1–2	0	32
	4	0	16
	5–6	0	32
	7–9	0	16
AKR/J	6	2	4
C57BL/6J	3	0	0
	7–8	0	0
	>12	0	0

*Antigen titer was determined by immunofluorescence absorption test of anti-p30 and anti-gp69/71 as described in the Materials and Methods. The residual antibody activity was tested on acetone-fixed E_5G2 cells.

determined by Ouchterlony tests. A precipitin line of identity was observed between concentrated NZB sera and sonicated Rauscher MuLV against anti-gp69/71, with faint spur formation indicating the presence of type-specific antibodies in the antiserum reaction with Rauscher MuLV. The precipitin reaction against anti-gp69/71 was also observed with concentrated sera of B/WF_1, NZW, and AKR/J. The precipitin reaction against anti-p30 was not detected even after the same sera had been treated with ether so as to disrupt any virus particles.

Immunofluorescence Analysis of Kidney Sections. Frozen sections of kidneys of NZB, B/WF_1, NZW, C57BL/6J, and AKR/J mice at various ages were analyzed by immunofluorescence for the location of viral envelope glycoprotein, gp69/71 and internal protein, p30. The bound immunoglobulins located in the kidneys were simultaneously studied by use of fluoresceinated goat antibody for the presence of mouse immunoglobulins.

With NZB mice, gp69/71 was found deposited with bound immunoglobulins,

mainly in the mesangia and less in the peripheral capillary loops and cytoplasm of some tubules (Figs. 1 and 2). The glomerular deposition of this protein in mice of different ages usually appeared to coincide with the manifestation of significant proteinuria and glomerulonephritis. The p30 protein was not detected in the kidneys over the age range studied.

With B/WF_1 mice, gp69/71 was found heavily deposited in a granular pattern, mainly in the peripheral capillary loops and less in the mesangia (Figs. 3 and 4). The distribution of gp69:71 was similar to that of bound immunoglobulins and was related to the severity of lupus-like glomerulonephritis (Fig. 5). A B/WF_1 mouse also revealed a granular deposition of both gp69/71 and bound immunoglobulins in the walls of vessels affected by an acute necrotizing vasculitis (Fig. 6). There was no specific deposition of p30 in the kidneys over the age range studied.

With NZW and C57BL/6J mice, gp69/71 and bound immunoglobulins were found to be deposited in the glomeruli, mainly in the mesangia and increasing with age (Figs. 7 and 8). The p30 protein was not demonstrated in the kidneys. A minimal histologic grade of nephritis was present in many of these mice.

With aging AKR/J mice, gp69/71 was present mainly in the mesangia with diffuse pattern (figure not presented). Bound immunoglobulins were detected focally in the mesangia, sometimes along capillary walls. A trace amount of p30 was detected in the glomeruli of both young and aging mice. A minimal grade of nephritis was noted in aging mice.

The immunofluorescence analysis of kidney sections by anti-NTD revealed that the fluorescence pattern of MuLV antigens detected by anti-NTD was quite similar to that detected by anti-gp69/71 serum, in keeping with the finding that the predominant viral protein in the tissues is gp69/71 (Fig. 9). The mice studied, the proteinuria status, and the extent of deposition of MuLV antigens and bound immunoglobulins are summarized in Table III.

Specificity of Immunofluorescence Reaction. The specificity of immunofluorescence reactions on the kidney sections was tested by absorbing the anti-gp69/71 with various viral antigens and mouse gamma globulins as previously described (25). The residual antibody activity was tested on the kidney sections of NZB, B/WF_1, and C57BL/6J mice, respectively. No immunofluorescence reaction was observed after absorption with either sonicated $E_{\delta}G2$ and AKR/J leukemic thymus, known to contain high concentrations of virus or viral proteins, or intact Rauscher MuLV suspension. Sonicated thymus of C57BL/6J mice, a low-leukemia low-virus strain, intact feline leukemia virus suspension, and mouse gamma globulins did not absorb out the antibody activity from anti-gp69/71 (Fig. 10). The results were fully concordant for NZB, B/WF_1, and C57BL/6J mice. It was concluded that the immunofluorescence reaction with anti-gp69/71 specifically involves the group-specific antibodies of the anti-Rauscher MuLV gp69/71 reacting with the related determinants of gp69/71 produced by the endogenous viruses of the other strains of mice tested.

Elution of Bound Immunoglobulins and Viral Antigens. Sections of kidneys were eluted with 0.12 M glycine buffer followed by immunofluorescence analysis which showed removal of both bound immunoglobulins and gp69/71 from the glomeruli of each of the positive kidneys except for strain AKR/J. The

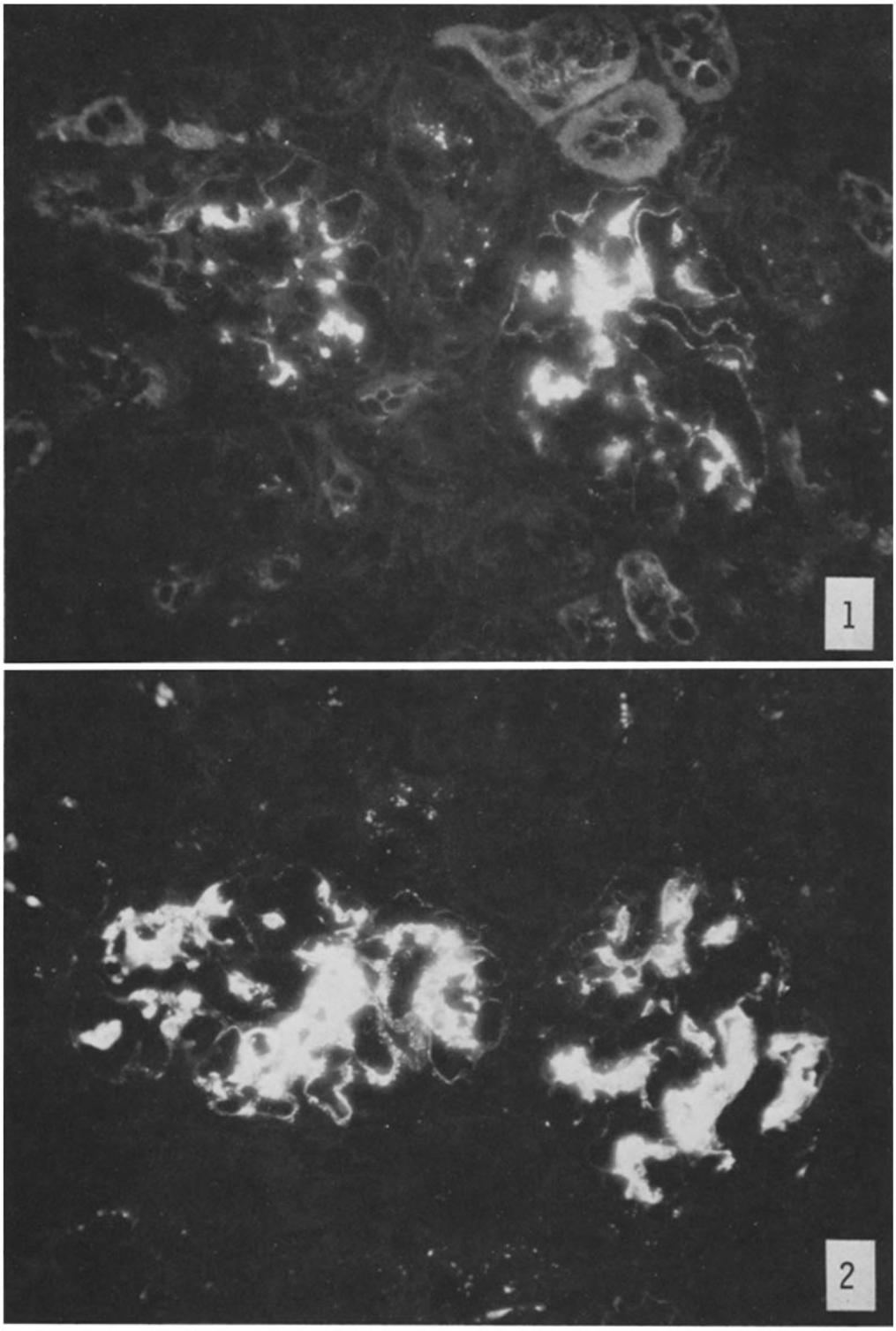
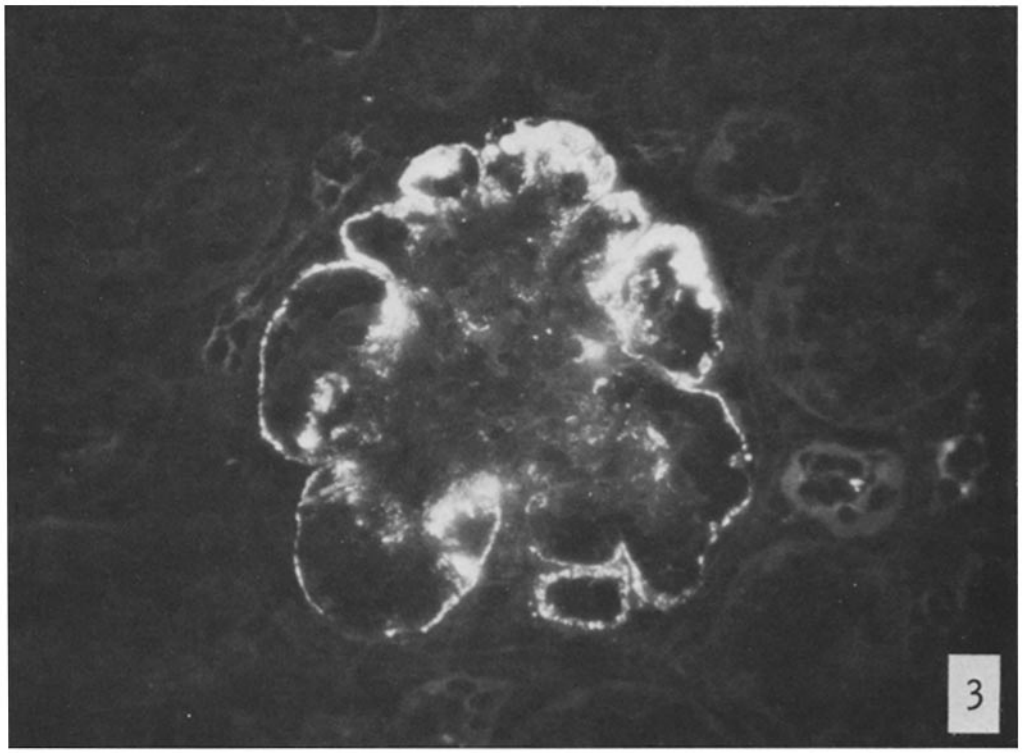


FIG. 1 and FIG. 2. Immunofluorescence photomicrographs of frozen section of perfused kidneys of mice. (1) NZB (12 mo, ♀). gp69/71 located in mesangia and wall of peripheral capillary loops of two glomeruli and also in some tubules. × 500. (2) Bound immunoglobulins located in mesangia and wall of peripheral capillary loops of two glomeruli in nearby section. × 500.



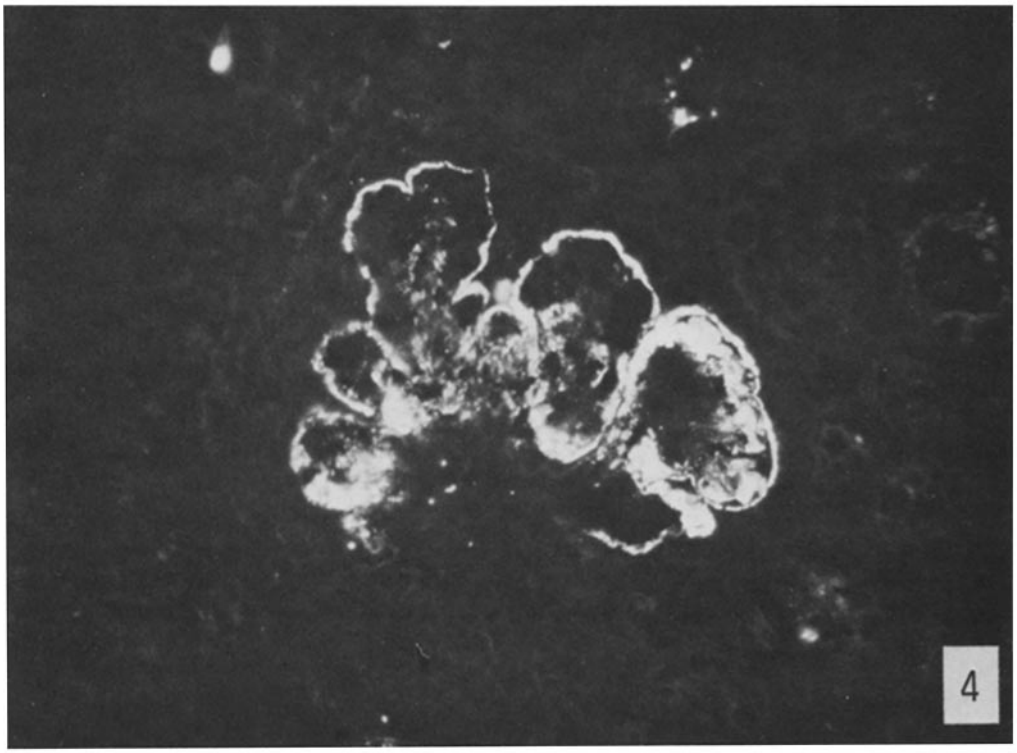


FIG. 3 and FIG. 4. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. B/WF$_1$ (12 mo, ♂). gp69/71 heavily located in wall of peripheral capillary loops and to a lesser extent in mesangia of two glomeruli and also in some tubules. × 500.

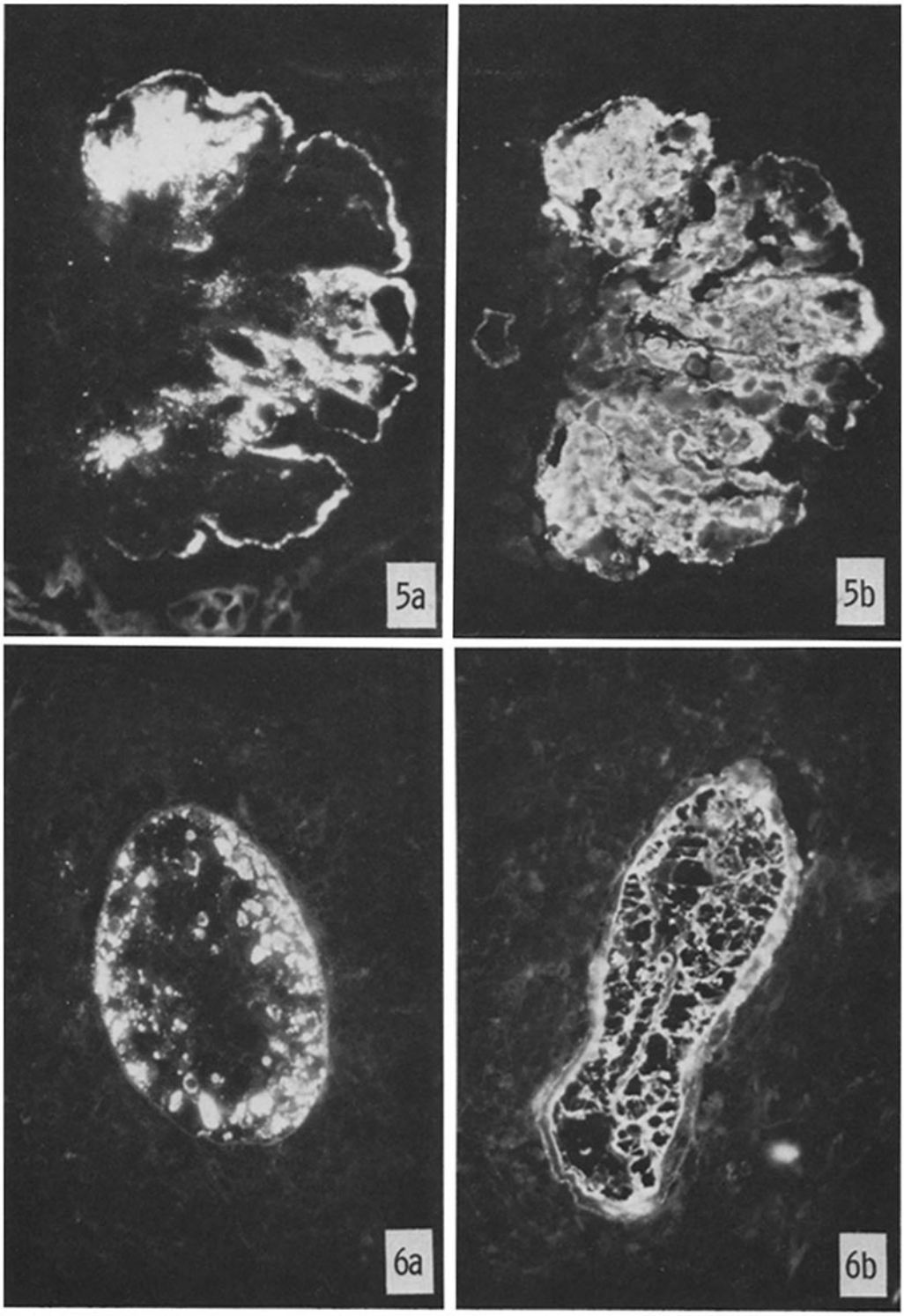


FIG. 5. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. (*a*) B/WF$_1$ (12 mo, ♂). gp69/71 located in a glomerulus. (*b*) Bound immunoglobulins located in the same glomerulus in nearby section. Note that the glomerular distribution of both gp69/71 and bound immunoglobulins is strikingly similar although the fluorescence pattern is different from one to the other. × 500.

FIG. 6. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. (*a*) B/WF$_1$ (14 mo, ♀). gp69/71 located in the wall of a vessel affected by an acute necrotizing vasculitis. × 415. (*b*) Bound immunoglobulins located in the same affected vessel in nearby section. Note similar distribution of gp69/71 and bound immunoglobulins. × 415.

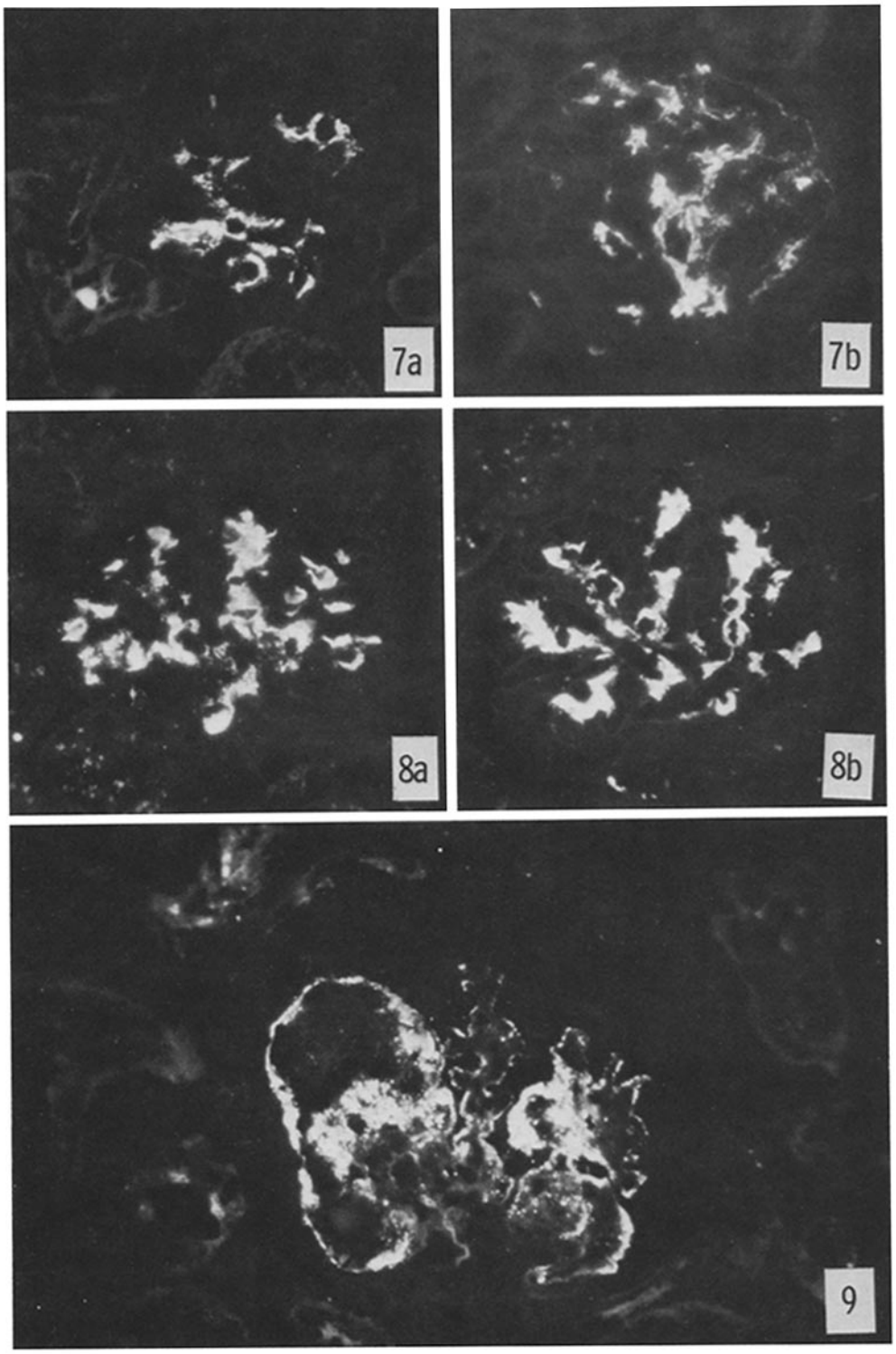


FIG. 7. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. (*a*) NZW (10 mo, ♀). gp69/71 located in mesangium and capillary wall of a glomerulus. × 500. (*b*) Bound immunoglobulins located in mesangium and sparsely in wall of peripheral capillary loops of a glomerulus in nearby section. × 500.

distribution of gp69/71 in the tubules was not changed. The results indicate that gp69/71 located in the glomeruli of these mice was deposited with bound immunoglobulins, whereas that in the tubules was neither associated with immunoglobulins nor eluted from the tubular cells. With AKR/J kidney sections, the detection of p30 and gp69/71 in the glomeruli was enhanced after elution of bound immunoglobulins, suggesting that in this strain the gp69/71 and p30 proteins were mainly firmly fixed to the glomeruli.

Demonstration of gp69/71 in the Kidney Eluate. The presence of gp69/71 in the kidney eluates was demonstrated by absorption of anti-gp69/71 using the kidney eluates as antigen source, and testing the residual antibody activity against acetone-fixed E♂G2 cells as described in the Materials and Methods. The relative amount of gp69/71 in the kidney eluate was related to the severity of glomerulonephritis. The gp69/71 antigen titer (starting dilution 1:4), strain or hybrid, and age were as follows: <4, C57BL/6J (8–12 mo), NZW (11–18 mo), AKR (6 mo); 4, B/WF$_1$ (3–4 mo); 8, NZB (9–22 mo); 16, B/WF$_1$ (5–8 mo). These results are consistent with the observed distribution of gp69/71 in the kidney sections and also with the disappearance from the glomeruli of both gp69/71 and bound immunoglobulins after glycine buffer elution.

Discussion

The main conclusions that can be drawn from these experiments are that the tissues and serum of NZB, NZW, and their F$_1$ hybrid mice contain a remarkably high concentration of the viral envelope glycoprotein, gp69/71, more than 10-fold greater than that found in the high incidence leukemia strain AKR, 30- to 40-fold greater than in the low incidence leukemia strain C57BL/6, and 1,000-fold greater than in BALB mice (30); and that the gp69/71 is deposited, apparently as an immune complex, in the diseased kidneys of these mice and in much greater concentration than the p30 major structural protein of the virus. While minor viral proteins not analysed in this study may also prove to be of pathogenetic importance, the present investigation suggests that the pathogenesis of immune complex glomerulonephritis in mice is in some manner related specifically to the expression of the glycoprotein and the host immune response to this protein. Several genes influence the relative expression of virus and viral proteins, Gv-1, *H-2*, or Fv-1 (see review, 31), and one or more of these genes may be abnormally expressed in New Zealand mice (30). Aging New Zealand mice are deficient in the T-cell-mediated immune response (32–38); it is possible that this abnormality is related to the extremely high concentration of gp69/71 in the tissues and serum of these mice (30, 39).

FIG. 8. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. (*a*) C57BL/6J (8 mo, ♀). gp69/71 located in mesangium of a glomerulus. × 500. (*b*) Bound immunoglobulins located in mesangium of a glomerulus in nearby section. × 500.

FIG. 9. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. B/WF$_1$ (12 mo, ♂). MuLV-related antigens located in a glomerulus demonstrated by anti-NTD. Note that the glomerular distribution and fluorescence pattern is similar to that of gp69/71 (see Figs. 3–5 *a*). × 500.

TABLE III

Mice Studied, Proteinuria Status, and Findings in Kidneys

Strain	Age	No. of mice	Protein-uria	Nephritis*	Host IgG‡	p30‡	gp69/71‡
	mo						
NZB	3	3	–	–	+	–	–
	6	3	–/+	+	+	–	–/+
	8	1	2+	2+	2+	–	2+
	9	2	2+/3+	2+/3+	2+/3+	–	2+/3+
	12	1	3+	2+	3+	–	3+
	13	3	3+	3+	2+/3+	–	2+/3+
	13	2	3+	3+	3+	–	–/+
	16	1	3+	3+	3+	–	2+
B/WF$_1$	2	4	–/+	–	–	–	–
	8	3	3+	3+	3+	–	3+
	11	3	3+	2+/3+	3+	–	2+/3+
	12	1	3+	3+	3+	–	3+
	14	1	3+	3+	3+	–	3+
NZW	3	3	–	–	–	–	–/tr.
	10	3	–/+	–/+	+	–	–/+
C57BL/6J	2	3	+	–	–	–	–
	8	3	–/+	+	+	–	+
AKR/J	2	3	+	–	–/tr.	–/tr.	–/tr.
	6	3	–/+	+	+	–/tr.	+

*The histologic grading of nephritis was maximal (3+), intermediate (2+), minimal (+), and negative (–).

‡The degree of fluorescence staining for host IgG, p30, and gp69/71 in the glomeruli was diffuse intense (3+), focal intense (2+), trace (tr.), and negative (–).

A clearly important factor in the pathogenesis of immune complex-type glomerulonephritis (40, 41) is the quantity and quality of the antibodies produced in the host immune response to specific antigenic stimulation. It is now evident that the immune response to a specific antigen is a complex process which involves host genetic controls at several levels. In mice, *Ir* (immune response) -1 genes linked to the K end of *H-2* locus are known to determine the extent of antibody response to certain antigens (42, 43). Sato et al. (44) have demonstrated that the immune response to MuLV envelope antigen is controlled by the Rgv-1 locus (31, 45) which is located at the K region of *H-2* and functions as an immune response gene. It appears that the immune response of NZB and NZW mice to the gp69/71 differs. Both strains have similar high tissue and serum concentration of this protein, yet the NZW strain develops a minimal histologic grade of nephritis compared to NZB and their F_1 hybrid mice. As antibodies to viral envelope components of MuLV have been described (9–11, 46, 47) and endogenous mouse antibodies specifically directed against gp69/71 have been detected (M. Strand and J. T. August, unpublished data), experiments to measure the amount of antibodies to gp69/71 in the kidney eluates and serum of

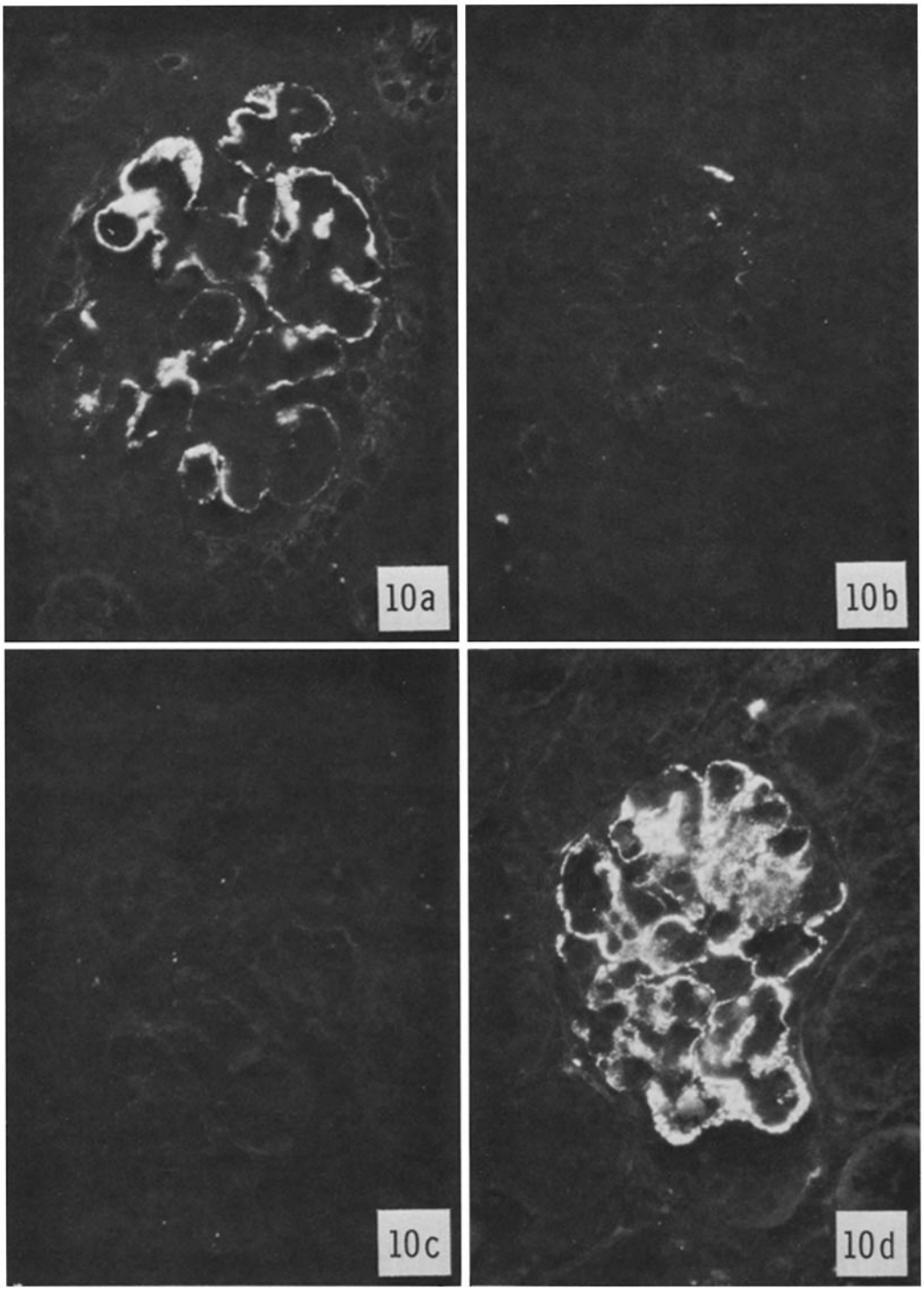


FIG. 10. Immunofluorescence photomicrographs of frozen sections of perfused kidneys of mice. B/WF$_1$ (11 mo, ♂). Specificity of immunofluorescence reaction by anti-gp69/71. (*a*) Anti-gp69/71 absorbed with sonicated C57BL/6J thymus. (*b*) Anti-gp69/71 absorbed with intact Rauscher MuLV suspension, (*c*) Anti-gp69/71 absorbed with sonicated E♂G2 cells. (*d*) Bound immunoglobulins located in wall of peripheral capillary loops and mesangium in section of same kidney. Note the specific absorption of the fluorescence reaction (*b* and *c*), and the similar distribution of gp69/71 and bound immunoglobulins (*a* and *d*). × 500.

these mice have been initiated. The relatedness of these findings to the nuclear antigen-antibody system that has been described (48, 49) is also yet to be determined.

With B/WF_1 mice, the gp69/71 was located predominantly in the wall of peripheral capillary loops, correlating with the severity of capillary damage. In contrast, with NZB mice, gp69/71 was mainly deposited in the mesangia, less in the peripheral capillary loops. These distribution patterns apparently correlated with the accelerated fatal clinical course of lupus-like glomerulonephritis in B/WF_1 mice and the slow progressive course of chronic glomerulonephritis in NZB mice. With AKR/J mice, the detection of gp69/71 and p30 in the glomeruli was enhanced after the elution of bound immunoglobulins, indicating that MuLV antigens in the glomeruli of AKR/J mice were mainly intrinsic to the glomeruli, most likely MuLV antigens formed by mesangial cells (50), and suggesting that locally formed as well as circulating and deposited (28, 51), MuLV antigens may be of importance in the pathogenesis of glomerulonephritis in AKR/J mice.

Summary

The use of monospecific antisera for the analysis by radioimmunoassay and immunofluorescence study of two major viral proteins, gp69/71 and p30 of murine leukemia virus, that could be of significance in the pathogenesis of immune complex glomerulonephritis of mice, particularly NZB and B/WF_1 hybrid mice, yielded the following conclusions. A remarkably high concentration of viral envelope glycoprotein, gp69/71, was detected in the spleen and serum of New Zealand mice (NZB, NZW, B/WF_1, and W/BF_1); the concentration in the spleen was 10-fold greater than that found in AKR mice and 30-fold greater than that present in C57BL/6 mice. The gp69/71 was deposited along with bound immunoglobulins, apparently as an immune complex, in the diseased kidneys of mice, and the glomerular site and extent of deposition of gp69/71 was related to the severity of the glomerulonephritis. This study suggests that the pathogenesis of immune complex glomerulonephritis (and vasculitis) in mice is related to the expression of this specific viral envelope glycoprotein and to the host immune response to this protein.

We acknowledge our indebtedness to Dr. Leonhard Korngold for helpful advice, Mrs. Phyllis Marinaccio for immunohistological work, Mr. Brent Keller for technical assistance, Mr. Louis Dienes for photomicrographic work, and Miss Eva Hochenburger for manuscript preparation.

Received for publication 9 May 1974.

References

1. Mellors, R. C. 1966. Autoimmune and immunoproliferative diseases of NZB mice and hybrids. *Int. Rev. Exp. Pathol.* **5**:217.
2. Howie, V. B., and B. J. Helyer. 1968. The immunology and pathology of NZB mice. *Adv. Immunol.* **9**:215.
3. East, J. 1970. Immunopathology and neoplasms in New Zealand Black (NZB) and SJL/J mice. *Prog. Exp. Tumor Res.* **13**:84.

4. Mellors, R. C., and C. Y. Huang. 1967. Immunopathology of NZB/BL mice. VI. Virus separable from spleen and pathogenic for Swiss Mice. *J. Exp. Med.* **126**:53.
5. Mellors, R. C., T. Aoki, and R. J. Huebner. 1969. Further implications of murine leukemia-like virus in the disorder of NZB mice. *J. Exp. Med.* **129**:1045.
6. Mellors, R. C., T. Shirai, T. Aoki, R. J. Huebner, and K. Krawczynski. 1971. Wild-type Gross leukemia virus and the pathogenesis of the glomerulonephritis of New Zealand mice. *J. Exp. Med.* **133**:113.
7. Mellors, R. C., and C. Y. Huang. 1966. Immunopathology of NZB/BL mice. V. Viruslike (filtrable) agent separable from lymphoma cells and identifiable by electron microscopy. *J. Exp. Med.* **124**:1031.
8. Mellors, R. C. 1968. Autoimmune disease and neoplasia of NZB mice-implication of murine leukemia-like virus. *Perspect. Virol.* **6**:239.
9. 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.
10. Aoki, T., and T. Takahashi. 1972. Viral and cellular surface antigens of murine leukemias and myelomas. *J. Exp. Med.* **135**:443.
11. Aoki, T., R. B. Herberman, P. A. Johnson, M. Liu, and M. M. Sturm. 1972. Wild-type Gross leukemia virus: classification of soluble antigen (GSA). *J. Viol.* **10**:1208.
12. Strand, M., and J. T. August. 1973. Structural proteins of oncogenic RNA viruses: interspec II, a new interspecies antigen. *J. Biol. Chem.* **248**:5627.
13. Strand, M., and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses: multiple antigenic determinants of the major internal protein and envelope glycoprotein. *J. Virol.* **13**:171.
14. Steeves, R. A., M. Strand, and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses: murine leukemia virus neutralization by antisera prepared against purified envelope glycoprotein. *J. Virol.* **14**:187.
15. Geering, G., L. J. Old, and E. A. Boyse. 1966. Antigens of leukemias induced by naturally occurring murine leukemia virus: their relation to the antigens of Gross virus and other murine leukemia virus. *J. Exp. Med.* **124**:753.
16. Geering, G., W. D. Hardy, Jr., L. J. Old, E. de Harven, and R. S. Brodey. 1968. Shared group-specific antigen of murine and feline leukemia viruses. *Virology*. **36**:678.
17. Geering, G., T. Aoki, and L. J. Old. 1970. Shared viral antigen of mammalian leukemia viruses. *Nature* (*Lond*). **226**:265.
18. Hardy, W. D., Jr., G. Geering, L. J Old, E. de Harven, R. S. Brodey, and S. K. McDonough. 1970. Serological studies of the feline leukemia virus. Comparative leukemia research. *Bibl. Haematol.* **36**:343.
19. Nowinski, R. C., L. J. Old, P. V. O'Donnell, and F. K. Sanders. 1971. Serological identification of Hamster oncornaviruses. *Nat. New Biol.* **230**:282.
20. Stockert, E., L. J. Old, and E. A. Boyse. 1971. The GIX system, a cell surface alloantigen associated with murine leukemia virus: implications regarding chromosomal integration of the viral genome. *J. Exp. Med.* **138**:1334.
21. Stockert, E., H. Sato, K. Itakura, E. A. Boyse, L. J. Old, and J. J. Hutton. 1973. Location of the second gene required for expression of the leukemia-associated mouse antigen GIX. *Science* (*Wash. D. C.*). **178**:862.
22. Yoshiki, T., R. C. Mellors, and W. D. Hardy, Jr. 1973. Common cell-surface antigen associated with murine and feline C-type RNA leukemia viruses. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1878.
23. Hunter, W. M. 1967. *In* Handbook of Experimental Immunology. D. M. Weir, editor, F. A. Davis Co., Philadelphia, Pa. 608–654.

24. Koffler, D., P. H. Schur, and H. G. Kunkel. 1967. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. Exp. Med.* **126**:607.
25. Yoshiki, T., R. C Mellors, W. D. Hardy, Jr., and E. Fleissner. 1974. Common cell surface antigen associated with mammalian C-type RNA viruses: cell membrane-bound gs antigen. *J. Exp. Med.* **139**:925.
26. Hilgers, J., R. C. Nowinski, G. Geering, and W. D. Hardy, Jr. 1972. Detection of avian and mammalian oncogenic RNA viruses (oncornaviruses) by immunofluorescence. *Cancer Res.* **32**:98.
27. Old, L. J., E. A. Boyse, and E. Stockert. 1965. The G (Gross) leukemia antigen. *Cancer Res.* **25**:813.
28. Oldstone, M. B. A, T. Aoki, and F. J. Dixon. 1972. The antibody response of mice to murine leukemia virus in spontaneous infection: absence of classical immunologic tolerance. *Proc. Natl. Acad. Sci. U. S. A.* **69**:134.
29. Mellors, R. C. 1965. Autoimmune disease in NZB/BL mice. I. Pathology and pathogenesis of a model system of spontaneous glomerulonephritis. *J. Exp. Med.* **122**:25.
30. Strand, M., F. Lilly, and J. T. August. 1974. Host control of endogenous murine leukemia virus gene expression: Concentrations of viral proteins in high and low leukemia mouse strains. *Proc. Natl. Acad. Sci. U. S. A.* In press.
31. Lilly, F., and T. Pincus. 1973. Genetic control of murine viral leukemogenesis. *Adv. Cancer Res.* **17**:231.
32. Cantor, H., R. Asofsky, and N. Talal. 1970. Synergy among lymphoid cells mediating the graft-versus-host response. I. Synergy in graft-versus-host reactions produced by cells from NZB/BL mice. *J. Exp. Med.* **131**:223.
33. Leventhal, B. G., and N. Talal. 1970. Response of NZB and NZB/NZW spleen cells to mitogenic agents. *J. Immunol.* **104**:918.
34. Rodney, G. E., R. A. Good, and E. J. Yunis. 1971. Progressive loss in vitro of cellular immunity with aging in strains of mice susceptible to autoimmune disease. *Clin. Exp. Immunol.* **9**:305.
35. Shirai, T., and R. C. Mellors. 1972. Natural cytotoxic autoantibody against thymocytes in NZB mice. *Clin. Exp. Immunol.* **12**:133.
36. Shirai, T., T. Yoshiki, and R. C. Mellors. 1972. Age-decrease of cells sensitive to an autoantibody-specific for thymocytes and thymus-dependent lymphocytes in NZB mice. *Clin. Exp. Immunol.* **12**:455.
37. Stutman, O. 1972. Lymphocyte subpopulation in NZB mice: deficit of thymus-dependent lymphocytes. *J. Immunol.* **109**:602.
38. 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.
39. Levy, J. A., 1973. Xenotropic viruses: murine leukemia viruses associated with NIH Swiss, NZB, and other mouse strains. *Science (Wash. D. C.).* **182**:1151.
40. Germuth, F. G., Jr., and E. Rodriguez. 1973. Immunopathology of the renal glomerulus. Immune complex deposit and antibasement membrane disease. Little, Brown and Company, Boston, Mass. 5-56.
41. Cochrane, C. G., and D. Koffler. 1973. Immune complex disease in experimental animals and man. *Adv. Immunol.* **16**:185.
42. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11**:31.
43. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.
44. Sato, H., E. A. Boyse, T. Aoki, C. Iritani, and L. J. Old. 1973. Leukemia-associated

transplantation antigens related to murine leukemia virus. The X.1 system: immune response controlled by a locus linked to *H-2*. *J. Exp. Med.* **138**:593.

45. Lilly, F. 1966. The inheritance of susceptibility to the Gross leukemia virus in mice. *Genetics*. **53**:529.
46. Batzing, B. L., M. Yurconic, Jr., and M. G. Hanna, Jr. 1974. Autogenous immunity to endogenous RNA tumor virus: chronic humoral immune response to virus envelope antigens in B6C3F_1 mice. *J. Natl. Cancer Inst.* **52**:117.
47. Ihle, J. N., M. G. Hanna, Jr., L. E. Roberson, and F. T. Kenney. 1974. Autogenous immunity to endogenous RNA tumor virus: Identification of antibody reactivity to select viral antigens. *J. Exp. Med.* **139**:1568.
48. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**:507.
49. Dixon, F. J., M. B. A. Oldstone, and G. Tonietti. 1971. Pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J. Exp. Med.* **134**(Suppl. 2):65s.
50. Pascal, R. R., M. N. Koss, and R. L. Kassel. 1973. Glomerulonephritis associated with immune complex deposits and viral particles in spontaneous murine leukemia: an electron microscopic study with immunofluorescence. *Lab. Invest.* **29**:159.
51. Oldstone, M. B. A., A. Tishon, G. Tonietti, and F. J. Dixon. 1972. Immune complex disease associated with spontaneous murine leukemia: incidence and pathogenesis of glomerulonephritis. *Clin. Immunol. Immunopathol.* **1**:6.