Proc. Natl. Acad. Sci. USA
Vol. 75, No. 5, pp. 2463–2467, May 1978
Medical Sciences

Type C RNA virus-specific antibody in human systemic lupus erythematosus demonstrated by enzymoimmunoassay

(proliferative glomerulonephritis/immune complex/viral core protein)

ROBERT C. MELLORS AND JANE W. MELLORS

The Hospital for Special Surgery, Affiliated with the New York Hospital-Cornell University Medical College, and the Departments of Pathology and Surgery, Cornell University Medical College, New York, New York 10021

Communicated by Walsh McDermott, March 3, 1978

ABSTRACT **Postmortem study of proliferative glomerulonephritis associated with human systemic lupus has previously shown that an antigen related to mammalian type C RNA viral core (p30) proteins is deposited in the renal glomerular lesions with human immunoglobulins in an immune-complex pattern. In the present work, human immunoglobulins were sequentially eluted from the lupus glomerular immune deposits and were assayed by a sensitive enzymoimmunoassay developed for the measurement of anti-p30 antibody activity against purified viral p30 proteins of mammalian type C viruses. Human immunoglobulins showing specific anti-p30 antibody activity, particularly against p30 antigen of feline endogenous virus RD-114 and to a smaller extent against p30 antigen of murine type C virus, were eluted by acid buffer from the glomerular immune deposits in two patients with lupus proliferative glomerulonephritis who have deposits of viral p30-related antigen in the same tissue lesions. This study adds support for the hypothesis that expression of type C viral antigen may be involved in the multifactorial pathogenesis of proliferative glomerulonephritis associated with human systemic lupus.**

Human systemic lupus erythematosus (SLE) is a multisystemic autoimmune syndrome of unknown etiology and multifactorial pathogenesis. Postmortem study of a subset of systemic lupus associated with proliferative glomerulonephritis has shown that an antigen related to the interspecies determinants of mammalian type C RNA viral core (p30) proteins is located in the renal glomerular lesions with human immunoglobulins (Igs) in an immune-complex pattern of deposition (1, 2). We attempt to extend this finding by examining the immune deposits for the presence of a type C virus antibody. Human Igs were sequentially eluted from the lupus glomerular immune deposits and were assayed for anti-p30 antibody activity against purified viral p30 protein antigens of mammalian type C viruses. A sensitive enzymoimmunoassay was developed for detection and measurement of anti-p30 antibodies. Human Igs showing specific anti-p30 antibody activity by this assay were eluted from the glomerular immune deposits in two patients with lupus proliferative glomerulonephritis known from previous work to contain deposits of viral p30-related antigen in the same tissue lesions. This investigation adds support for the hypothesis, stemming from studies of the murine model of systemic lupus (3, 4), that expression of type C viral antigen may be involved in the multifactorial pathogenesis of proliferative glomerulonephritis associated with human systemic lupus.

MATERIALS AND METHODS

Viruses. The following viruses purified by twice banding in sucrose gradients were provided by S. A. Mayyasi and K. A. Traul (John L. Smith Memorial for Cancer Research) through the courtesy of J. Gruber, Chief, Office of Program Resources and Logistics, National Cancer Institute: Rauscher murine leukemia virus (R-MuLV) propagated in mouse cell line JLSV9; feline leukemia virus, Theilen strain (FeLV) grown in feline cell line FL-74; and feline endogenous virus RD-114 propagated in human cell line RD.

Purified Viral Core (p30) Proteins. R-MuLV p30 and FeLV p27 proteins were kindly provided by S. Oroszlan (Flow Laboratories, Inc.). RD-114 p28 protein was the generous gift of C. J. Sherr (National Cancer Institute). Core proteins of murine mammary tumor virus (MMTV) and avian myeloblastosis virus (AMV), MMTV p28 and AMV p27, were kindly provided by D. P. Bolognesi (Duke University Medical Center).

Cells. Canine thymus-derived cell line FCf2Th infected with RD-114 virus, human cell line RD infected with RD-114, and FCf2Th cells infected with simian sarcoma associated virus (SSAV) were obtained through the courtesy of C. J. Sherr (National Cancer Institute) and were maintained in this laboratory by A. P. Albino. Feline lung fibroblasts (FLF) infected with FeLV were kindly provided by W. D. Hardy, Jr. (Memorial Sloan-Kettering Cancer Center). Mouse cells EδG_2 producing Gross leukemia virus (G-MuLV) and C1498 cells are standard transplantable leukemias of C57BL/6 mice.

Antisera. The following monospecific goat antisera produced against purified viral p30 proteins were used: anti-R-MuLV p30, anti-RD-114 p28, and anti-SSAV p28 serum obtained from R. Wilsnack (Huntingdon Research Center) through the courtesy of J. Gruber, National Cancer Institute; and anti-FeLV p27 serum kindly provided by S. Oroszlan (Flow Laboratories, Inc.). Normal goat serum and fetal calf serum were obtained from commercial sources.

Elution of Human SLE Kidney Igs. The starting materials were human renal tissues obtained at postmortem examination of three patients with SLE described elsewhere (1, 2). Briefly summarized, the duration of the disease in patients 1 and 2 was ½ and 2 years, respectively; the SLE was associated with diffuse proliferative glomerulonephritis and showed typical glomerular deposits of host Ig and, less extensively, of type C viral p30-like antigen; the duration of the disease in patient 3 was 15 years; the SLE was not associated with proliferative-type glomerulonephritis and showed glomerular deposits of host Ig but no detectable p30-like antigen. In the latter respect, patient 3 served as a negative control for the other two patients. The renal tissue was minced and homogenized in cold isotonic saline and the insoluble sediment containing glomeruli was thoroughly

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MuLV, murine leukemia virus; FeLV, feline leukemia virus; SSAV, simian sarcoma associated virus; RD-114, feline endogenous virus RD-114; MMTV, murine mammary tumor virus; AMV, avian myeloblastosis virus; SLE, systemic lupus erythematosus; P_i/NaCl, phosphate-buffered saline.

Table 1. Fluorescent antibody titers (endpoint dilutions) of goat anti-p30 sera against homologous and heterologous viral p30 antigen in acetone-fixed target cells

Cells	Infecting virus	Anti-R-MuLV p30	Anti-FeLV p27	Anti-RD-114 p28	Anti-SSAV p28
EϑG2	G-MuLV	2560	640–1280	40	40
FLF	FeLV	640	2560–5120	40	20
FCf2Th	RD-114	80–160	640–1280	320–640	40
RD	RD-114	80–160	1280	320	NT*
FCf2Th	SSAV	160	320	40	1280
Control†	—	<20	<20	<20	<20

* Not tested.
† Uninfected C1498, FLF, FCf2Th, and RD cells.

washed in saline. Host-bound Igs were sequentially eluted from the glomeruli in two fractions by the method of Koffler *et al.* (5): first, by using purified bovine DNase (Worthington Biochemical Corp.) to elute anti-DNA antibodies bound to DNA antigen and usually concentrated in the glomerular immune deposits in human SLE (5); and next, by using acid buffer (0.2 M glycine·HCl, pH 2.4) to elute any remaining antibodies, including those that might bind to other antigens, in the immune deposits in SLE (5). The Ig-containing DNase and acid buffer eluates so obtained were precipitated at 4° with an equal volume of saturated ammonium sulfate. The precipitates were centrifuged, washed, and reconstituted in 10 mM phosphate-buffered (pH 7.6) saline (P_i/NaCl) containing 1 mM EDTA, followed by dialysis and concentration to a small volume in an Amicon stirred cell with PM-10 membrane. Aliquots were frozen and stored at −70°. Protein concentration was determined by the standard Lowry method, with crystalline bovine serum albumin as reference standard. IgG, IgA, and IgM were measured by quantitative radial immunodiffusion with appropriate low level standards.

Immunofluorescence Analysis. For antibody tests, eluates were diluted in P_i/NaCl containing crystalline bovine serum albumin, 2 mg/ml, without or with addition of the proteinase inhibitor, Trasylol (Mobay Chemical Corp.), which did not affect the results in any way. Anti-nuclear antibody was titrated by indirect immunofluorescence with alcohol-fixed guinea-pig liver sections and fluorescein-labeled goat antibody to human Ig (which reacted with Ig classes G, A, and M), along with DNase-treated sections for proof of anti-nuclear antibody specificity. Goat anti-p30 sera was titrated by indirect immunofluorescence against p30 antigens as described (1, 6), with acetone-fixed virus-infected and uninfected target cells (Table 1) and fluorescein-labeled rabbit antibody to goat IgG. These target cells were also used for titration of anti-cytoplasmic antibody in eluates by indirect immunofluorescence with fluorescein-labeled goat antibody to human Ig and for reciprocal blocking tests with eluates and anti-p30 sera (see Table 3), wherein the starting concentration of anti-p30 serum was 2 or more times the endpoint dilution.

Enzymoimmunoassay. A sensitive enzymoimmunoassay, based on the microplate modification (7) of the enzyme-linked immunosorbent assay of Engvall and Perlmann (8), was developed for detection and measurement of anti-p30 antibody activity against purified mammalian type C viral p30 antigens. The general procedure for determination of antibodies by indirect enzymoimmunoassay (7, 8) involves adsorption of antigen to solid phase (wells of a microtiter plate); incubation with antiserum; after washings, addition of enzyme-conjugated anti-Ig and enzyme substrate; and timed measurement of label uptake by the solid phase, which measures the concentration of specific antibodies (detectable in the ng/ml range). In the present work, antigen was adsorbed to the polystyrene microplate at 4° for at least 72 hr; the optimal concentration of purified viral p30 antigen was 1–2 μg of p30 protein (in 10-fold this concentration of carrier bovine serum albumin) per ml of coating buffer. The goat anti-p30 sera and normal control goat serum were serially diluted in P_i/NaCl/Tween containing 0.5% bovine serum albumin. The working dilution of rabbit anti-goat IgG labeled with alkaline phosphatase (Sigma type VII) was 1:200. The enzyme substrate was *p*-nitrophenyl phosphate (Sigma 104 phosphate substrate). Absorbance was measured in a Gilford spectrophotometer at 400 nm. Eluates of human SLE kidney Ig and normal control human Ig were serially diluted in P_i/NaCl/Tween containing 0.5% bovine serum albumin and similarly assayed for reactivity against purified viral p30 protein antigens by enzymoimmunoassay developed with 1:150 dilution of alkaline phosphatase-labeled goat anti-human Ig (broadly reactive with human IgG, IgA, and IgM). The following test materials were also used as negative control antigens dissolved in coating buffer: AMV p27, MMTV p28, denatured calf thymus DNA, and poly(I·C), each one at 2 μg/ml in 10-fold this concentration of carrier bovine serum albumin; and fetal calf serum at 1:100–1:10,000 dilutions.

RESULTS

Type C Virus Antibody-Like Activity in Human SLE Kidney Eluates Determined by Immunofluorescence. The total protein concentration, host Ig concentration by class, and antibody activity by immunofluorescence analysis of DNase and acid eluates of human SLE kidney Ig are given in Table 2 for patients 1, 2, and 3. The Ig concentration in similarly concentrated eluates was 62–130 μg/ml, greater in the DNase than in the acid eluate in two of three patients and predominantly IgG. DNase eluates 1, 2, and 3 contained anti-nuclear antibody with anti-DNA specificity and high specific activity when expressed as minimum Ig concentration required for a positive reaction compared to serum from the same patient, in keeping with the work of Koffler *et al.* (5). Of the eluates and sera tested, only acid eluates 1 and 2 contained anti-cytoplasmic antibody. This antibody gave a similar titer (Table 2) and homogeneous pattern of cytoplasmic reaction with either virus-infected or uninfected target cells listed in Table 1. This result was consistent with the finding that some kidney eluates and sera of human SLE patients contain antibodies against ribonucleoproteins (5) and cytoplasmic antigens of normal cells (9, 10). Nevertheless, the specificity of the cytoplasmic reaction between virus-infected target cells and acid eluates was further analyzed by reciprocal blocking tests with goat anti-p30 sera (Table 3). Pretreatment of FCf2Th (RD-114) target cells with acid eluates 1 or 2 diluted up to 4-fold completely blocked the cytoplasmic reaction with anti-RD-114 p28 serum (Fig. 1), whereas pretreatment of the cells with anti-RD-114 p28 serum did not block the reaction with the eluates. The simplest in-

Table 2. Human Ig concentration and anti-nuclear and anti-cytoplasmic fluorescent antibody activity of eluates of human SLE kidney Ig compared to SLE sera

Eluate or serum	Protein, mg/ml	Ig, μg/ml				Antibody activity		
		Ig*	IgG	IgA	IgM	Specificity	Titer†	Min. Ig,‡ μg/ml
DNase 1	10.3	130	94	36	<20	Anti-nuclear	16–32	4–8
Acid 1	2.9	104	84	20	<20	Anti-cytoplasmic	8–16	7–13
DNase 2	12.8	82	60	22	<20	Anti-nuclear	4	20
Acid 2	2.9	100	82	18	<20	Anti-cytoplasmic	8	12
DNase 3	5.1	126	62	37	27	Anti-nuclear	32	4
						Anti-cytoplasmic	<1	>126
Acid 3	1.4	62	42	20	<20	Anti-nuclear	<1	>62
						Anti-cytoplasmic	<1	>62
Serum 1	38.5	3940	2460	1080	400	Anti-nuclear	32	123
						Anti-cytoplasmic	<1	>3940
Serum 2	49.5	8700	5400	2300	1000	Anti-nuclear	32	272
						Anti-cytoplasmic	<1	>8700

* Ig equals the sum of IgG, IgA, and IgM.
† Titer of antibody is reciprocal of highest dilution giving positive reaction with target antigens: liver cells for anti-nuclear, FCf2Th (RD-114) cells for anti-cytoplasmic reactivity.
‡ Minimum Ig required for positive reaction equals Ig divided by titer.

terpretation of this result was that the eluates contained antibodies to RD-114 p28 as well as antibodies to other viral or nonviral (cellular) antigens that cannot be blocked with anti-RD-114 p28 serum. The one-way blocking activity was removed by treating the eluates with anti-human Ig serum, but not with anti-human albumin serum; the blocking activity of the eluates was also removed by absorbing the eluates with disrupted RD-114 virus (data not shown). Pretreatment of FCf2Th (RD-114) cells with acid eluate 3, DNase eluates, and sera listed in Table 2, even when undiluted, did not block the cytoplasmic reaction with anti-RD-114 p28 serum. The specificity of the Ig-dependent, one-way blocking activity of acid eluates 1 and 2 was further analyzed with other virus-infected target cells and homologous anti-p30 sera. Pretreatment with acid eluates 1 or 2, even when undiluted, showed at most only weak blocking activity against the cytoplasmic reaction of FCf2Th (SSAV) cells with anti-SSAV p28 serum, of FLF (FeLV) cells with anti-FeLV p27 serum, and of EδG2 (G-MuLV) cells with anti-R-MuLV p30 serum. The interpretation of these results was that the blocking activity of acid eluates 1 and 2 was selective for p30 antigen in cells infected with RD-114 virus. The specificity of this type C virus antibody-like activity was further analyzed by enzymoimmunoassay.

Table 3. Cytoplasmic immunofluorescence reaction of FCf2Th(RD-114) target cells with acid eluates of human SLE kidney Ig from patients 1 and 2 and goat anti-RD-114 p28 serum in reciprocal blocking tests

Blocking reagent	Anti-p30 serum or eluate	Fluorescent conjugate	Observed immuno-fluores-cence	Blocking activity
	Acid eluate	Anti-human Ig	+	
	Anti-RD-114 p28	Anti-goat Ig	+	
Acid eluate	Anti-RD-114 p28	Anti-goat Ig	−	Complete
Anti-RD-114 p28	Acid eluate	Anti-human Ig	+	None
DNase eluate	Anti-RD-114 p28	Anti-goat Ig	+	None

Type C Viral p30 Antibody Activity of Reference Goat Anti-30 Sera Determined by Enzymoimmunoassay. The straight-line portion of the titration curve of dilutions, $\log_{10}(1/D)$, of reference goat anti-p30 sera against homologous type C viral p30 protein antigens as determined by enzymoimmunoassay and measurement of absorbance at 400 nm for anti-R-MuLV p30, anti-FeLV p27, and anti-RD-114 p28 sera are given in Fig. 2A. Antibody titers of these antisera against homologous and heterologous viral p30 antigens as determined by enzymoimmunoassay are given in Table 4 as endpoint dilutions giving an absorbance of 0.5, compared to a background of 0.1–0.2 at most. As noted in Table 4, the $\log_{10}$ titer of anti-R-MuLV p30 serum against R-MuLV p30 antigen was 6.0, against FeLV p27 it was 5.3, and against RD-114 p28 it was 5.0, corresponding to antiserum dilutions of 1,000,000, 200,000, and 100,000, respectively. The $\log_{10}$ titer of anti-RD-114 p28 serum against RD-114 p28 was 4.8 and against R-MuLV p30 or FeLV p27 it was 4.0, corresponding to antiserum dilutions of 60,000 and 10,000, respectively. These

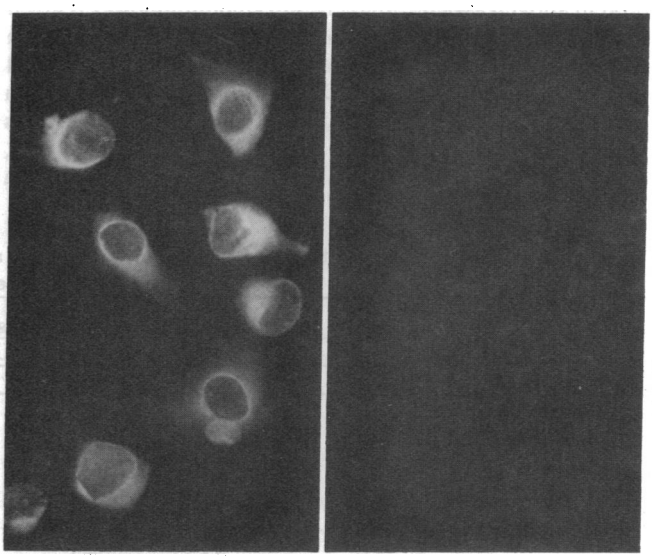

FIG. 1. Cytoplasmic immunofluorescence reaction between FCf2Th (RD-114) target cells and goat anti-RD-114 p28 serum (*Left*) is completely blocked by pretreatment of target cells with acid eluate of human SLE kidney Ig from patient 2 (*Right*). (×320.)

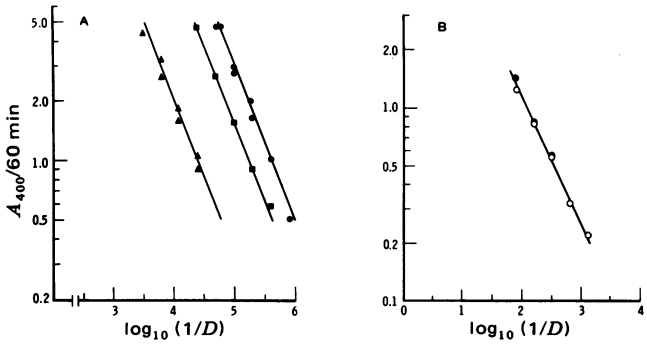

FIG. 2. (*A*) Straight-line portion of titration curve of dilutions, $\log_{10}(1/D)$, of goat anti-R-MuLV p30 (●), anti-FeLV p27 (■), and anti-RD-114 p28 (▲) sera against homologous viral p30 protein antigens as determined by enzymoimmunoassay and measurement of absorbance at 400 nm (A_{400}/60 min). (*B*) Straight-line portion of titration curve of dilutions, $\log_{10}(1/D)$, of acid eluates of human SLE kidney Ig from patients 1 (●) and 2 (○) against purified RD-114 p28 protein antigen as determined by enzymoimmunoassay and measurement of absorbance at 400 nm.

anti-p30 sera similarly diluted did not react with fetal calf serum or with any other control antigen tested, including the core protein of avian type C virus (AMV p27) or of murine type B virus (MMTV p28), denatured DNA, and poly(I·C) (data not shown). Normal goat serum even at 100-fold dilution did not react with the mammalian type C viral p30 protein antigens. The reactivity of each anti-p30 serum with the homologous p30 protein was removed by absorption with the same antigen (preliminary data not shown). It was concluded that enzymoimmunoassay was a sensitive, specific, and quantitative method for detection and measurement of mammalian type C virus p30 group-specific and interspecies-specific (cross-reacting) antibodies (11–15) in the reference antisera and that the minimum antibody detection limits indicated by the titers were comparable to, if not better than, those reported for similar antisera by radioimmunoassays (16–22).

Type C Viral p30 Antibody Activity of Human SLE Kidney Eluates Determined by Enzymoimmunoassay. The straight-line portion of the titration curve of dilutions, $\log_{10}(1/D)$, of acid eluates 1 and 2 against purified RD-114 p28 protein antigen as determined by enzymoimmunossay is given in Fig. 2*B* for doubling dilutions from 80 to 1280. This titration curve was parallel to that given by reference goat anti-RD-114 p28 serum against the same RD-114 p28 antigen but, as shown by comparison of Fig. 2 *A* and *B*, dilution points for the eluates were about 2 $\log_{10}$ units less than for the reference antiserum, in line with the relatively small starting concentration of Ig in eluates. The titer of eluates 1 and 2, expressed as the dilution giving an absorbance of 0.5, was 320 against RD-114 p28 (Table 5), whereas a 20-fold dilution of acid eluate 3 or DNase eluate did not react with this antigen. The titer of eluate 2 was 80 against purified R-MuLV p30 antigen and, again, a 20-fold dilution of acid eluate 3 or DNase eluate did not react with this antigen. Acid eluate 2 at a 20-fold dlution did not react by enzymoimmunoassay with any control antigens tested, including fetal calf serum, core protein of avian type C virus (AMV p27) and murine type B virus (MMTV p28), denatured DNA, and poly(I·C) (Table 5). Purified normal control human IgG at 5 μg/ml, equivalent to a 20-fold dilution of acid eluate 2, did not react by enzymoimmunossay with purified RD-114 p28 or R-MuLV p30. We concluded that acid eluates 1 and 2 contained a mammalian type C virus-specific antibody that, as determined by enzymoimmunoassay compared to reference anti-p30 sera, reacted strongly with RD-114 p28 antigen and to a smaller extent with R-MuLV p30 antigen, consistent with weaker crossreaction with a more distantly related type C virus group. The activity against RD-114 p28 was removed by absorbing the eluate with this antigen (unpublished).

DISCUSSION

Two chief conclusions can be drawn from these experiments. First, a sensitive, specific, and quantitative enzymoimmunoassay (8, 7), which compared favorably with radioimmunoassay (16–22), was developed for the detection and measurement of anti-p30 antibodies against p30 (core) protein antigens of mammalian type C RNA viruses. Second, autologous human Igs showing specific anti-p30 antibody activity against purified p30 antigens of mammalian type C viruses, but not against p30 antigens of avian type C or murine type B viruses, were eluted from the renal glomerular lesions in two patients with SLE. The autologous anti-p30 antibody was eluted by acid buffer from immune deposits located in renal glomeruli that were the site of lupus diffuse proliferative glomerulonephritis and that were known from previous work to contain an antigen related to the p30 proteins of mammalian type C viruses (1, 2). The human anti-p30 antibody had a titer of 320 determined by enzymoimmunoassay against purified RD-114 p28 protein, the major core protein of endogenous feline virus RD-114 which is antigenically related to, but distinguishable from, that of endogenous primate (baboon) type C viruses (19, 22). The human anti-30 antibody had a titer of 80 against purified R-MuLV p30 protein, consistent with weaker crossreaction with a more distantly related type C virus group (16–22). Preliminary assays of the antibody activity against SSAV p28 protein also gave a titer of 80 (unpublished work). Detection of autol-

Table 4. Antibody titers (endpoint dilutions) of goat anti-p30 sera against homologous and heterologous viral p30 antigens as determined by enzymoimmunoassay

	Goat antiserum							
	Anti-R-MuLV p30		Anti-FeLV p27		Anti-RD-114 p28		Normal goat serum	
p30 antigen	$\log_{10}(1/D)$	A_{400}	$\log_{10}(1/D)$	A_{400}	$\log_{10}(1/D)$	A_{400}	$\log_{10}(1/D)$	A_{400}
R-MuLV	5.6	1.0	4.5	1.0	3.6	1.0	2	<0.1
	6.0	*0.5*	*4.9*	*0.5*	*4.0*	*0.5*	2	<0.1
FeLV	4.9	1.0	5.2	1.0	3.6	1.0	2	<0.1
	5.3	*0.5*	*5.6*	*0.5*	*4.0*	*0.5*	2	<0.1
RD-114	4.6	1.0	NT‡	NT	4.4	1.0	2	<0.1
	5.0	*0.5*	NT	NT	*4.8*	*0.5*	2	<0.1
FCS*	2.0	<0.1	2.0	<0.1	2.0	<0.1	NT	NT

Italicized values, $\log_{10}(1/D)$, reciprocal dilution. A_{400}, enzyme–substrate incubation for 60 min. NT, not tested.
* Fetal calf serum. 1:100, 1:1000, 1:10,000.

Table 5. Specific anti-p30 antibody activity in acid eluates of human SLE kidney Ig from patients 1 and 2 by enzymoimmunoassay

Antigen	Antibody source	1/D	A_{400}/60 min
RD-114 p28	Acid eluate 1	160	0.85
	Acid eluate 1	320	0.56
	Acid eluate 2	160	0.83
	Acid eluate 2	320	0.56
	Acid eluate 2	640	0.32
	Acid eluate 2	1280	0.22
	DNase eluate 2	20	<0.10
	Acid eluate 3	20	<0.10
R-MuLV p30	Acid eluate 2	80	0.53
	Acid eluate 2	160	0.33
	Acid eluate 2	320	<0.10
	DNase eluate 2	20	<0.10
	Acid eluate 3	20	<0.10
MMTV p28	Acid eluate 2	20	<0.10
AMV p27	Acid eluate 2	20	<0.10
Denatured DNA	Acid eluate 2	20	<0.10
Poly(I·C)	Acid eluate 2	20	<0.10

ogous anti-p30 antibody and viral p30-related antigen in the tissue lesions supports the hypothesis, stemming from studies of the New Zealand mouse model of SLE (3, 4), that expression of type C viral antigens may be involved in the multifactorial pathogenesis of immune-deposit type proliferative glomerulonephritis associated with human systemic lupus. Nevertheless, the problem addressed here is a very controversial one. Other workers have used mammalian, including endogenous primate-related (23–25), type C viral nucleic acids (23, 26, 27) and p30 proteins (24, 25, 28) as probes for subinfectious expression of type C virus information in human tissues in cancerous conditions (23–28) and systemic lupus (25) and have reported variously some positive (23–27) or exclusively negative (28) results. Assays for human antibodies against mammalian, including endogenous primate-related (29, 30), type C viruses and viral proteins have also yielded some positive (29, 30), tentatively positive (31, 32), and exclusively negative (28, 33) tests on normal human sera and sera from patients with cancer or systemic lupus. Also to be noted are positive results of other studies reporting a putative type C viral antigen or antibody in human leukemia (34) and systemic lupus (35, 36). While some of the foregoing studies have provided evidence for subinfectious viral expression, a unique human type C virus has not yet been isolated, and candidate human virus isolates to date have been closely related to known endogenous and infectious subhuman primate type C viruses (27, 37–39).

We express appreciation to: J. Gruber, Office of Program Resources and Logistics, National Cancer Institute for reagents; C. J. Sherr, S. Oroszlan and D. P. Bologensi for purified viral proteins; R. Wilsnack and S. Oroszlan for antisera; S. A. Mayyasi and K. A. Traul for viruses; C. J. Sherr, A. P. Albino, W. D. Hardy, Jr. and M. Imamura for cells; N. Blumenthal and Miss E. Evans for use of the electrobalance. This work, Research Division Publication no. 611, was supported by Grant CA-14928 from the National Cancer Institute.

1. Mellors, R. C. & Mellors, J. W. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 233–237.
2. Mellors, R. C. & Mellors, J. W. (1978) *Arthritis Rheum.*, in press.
3. Mellors, R. C., Aoki, T. & Huebner, R. J. (1969) *J. Exp. Med.* **129**, 1045–1062.
4. Yoshiki, T., Mellors, R. C., Strand, M. & August, J. T. (1974) *J. Exp. Med.* **140**, 1011–1027.
5. Koffler, D., Agnello, V. & Kunkel, H. G. (1973) *Am. J. Pathol.* **74**, 109–124.
6. Hilgers, J., Nowinski, R. C., Geering, G. & Hardy, W. D., Jr. (1972) *Cancer Res.* **32**, 98–106.
7. Voller, A., Bidwell, D. & Bartlett, A. (1976) in *Manual of Clinical Immunology*, eds. Rose, N. R. & Friedman, H. (American Society for Microbiology, Washington, DC), pp. 506–512.
8. Engvall, E. & Perlmann, P. (1972) *J. Immunol.* **109**, 129–135.
9. Clark, G., Reichlin, M. & Tomasi, T. B., Jr. (1969) *J. Immunol.* **102**, 117–122.
10. Mattioli, M. & Reichlin, M. (1974) *Arthritis Rheum.* **17**, 421–429.
11. Huebner, R. J., Armstrong, D., Okuyan, M., Sarma, P. S. & Turner, H. C. (1964) *Proc. Natl. Acad. Sci. USA* **51**, 742–750.
12. Geering, G., Old, L. J. & Boyse, E. A. (1966) *J. Exp. Med.* **124**, 753–772.
13. Geering, G., Hardy, W. D., Jr., Old, L. J. & de Harven, E. (1968) *Virology* **36**, 678–707.
14. Gilden, R. V., Oroszlan, S. & Huebner, R. J. (1971) *Nature New Biol.* **231**, 107–108.
15. August, J. T., Bolognesi, D. P., Fleissner, E., Gilden, R. V. & Nowinski, R. C. (1974) *Virology* **60**, 595–601.
16. Oroszlan, S., Huebner, R. J. & Gilden, R. V. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 901–904.
17. Parks, W. P. & Scolnick, E. M. (1973) *Proc. Natl. Acad. Sci. USA* **69**, 1766–1770.
18. Stephenson, J. R. & Aaronson, S. A. (1973) *J. Virol.* **12**, 564–569.
19. Scherr, C. J. & Todaro, G. J. (1974) *Virology* **61**, 168–181.
20. Gilden, R. V., Toni, R., Hanson, M., Bova, D., Charman, H. P. & Oroszlan, S. (1974) *J. Immunol.* **112**, 1250–1254.
21. Strand, M. & August, J. T. (1975) *J. Virol.* **15**, 1332–1341.
22. Sherr, C. J., Fedele, L. A., Benveniste, R. E. & Todaro, G. J. (1975) *J. Virol.* **15**, 1440–1448.
23. Benveniste, R. A. & Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4513–4518.
24. Sherr, C. J. & Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4703–4707.
25. Strand, M. & August, J. T. (1974) *J. Virol.* **14**, 1584–1596.
26. Hehlmann, R., Kufe, D. & Spiegelman, S. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 435–439.
27. Reitz, M. S., Miller, N. R., Wong-Staal, F., Gallagher, R. E., Gallo, R. C. & Gillespie, D. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2113–2117.
28. Stephenson, J. R. & Aaronson, S. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1725–1729.
29. Aoki, T., Walling, M. J., Bushar, G. S., Liu, M. & Hsu, K. C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2491–2495.
30. Kurth, R., Teich, N. M., Weiss, R. & Oliver, R. T. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1237–1241.
31. Snyder, W. H., Jr., Pincus, T. & Fleissner, E. (1976) *Virology* **75**, 60–73.
32. Louie, S., Curtis, T. E., Till, J. E. & McCulloch, E. A. (1976) *J. Exp. Med.* **144**, 1243–1253.
33. Charman, H. P., Kim, N., White, M. & Gilden, R. V. (1974) *J. Natl. Cancer Inst.* **52**, 1409–1413.
34. Sutherland, J. C. & Mardiney, M. R., Jr. (1973) *J. Natl. Cancer Inst.* **50**, 633–644.
35. Lewis, R. M., Tannenberg, W., Smith, C. & Schwartz, R. S. (1975) *Nature* **252**, 78–79.
36. Panem, S., Ordonez, N. G., Kirsten, W. H., Katz, A. I. & Spargo, B. H. (1976) *N. Engl. J. Med.* **295**, 470–475.
37. Gallagher, R. E. & Gallo, R. C. (1975) *Science* **187**, 350–353.
38. Panem, S., Prochownik, E. V., Reale, F. R. & Kirsten, W. H. (1975) *Science* **189**, 297–299.
39. Nooter, K., Aarssen, A. M., Bentvelzen, P., deGroot, F. G. & Van Pelt, F. G. (1975) *Nature* **256**, 595–597.