THE EFFECT OF INDUCED CHRONIC VIRAL INFECTIONS ON THE IMMUNOLOGIC DISEASES OF NEW ZEALAND MICE*

By GIORGIO TONIETTI‡, M.D., MICHAEL B. A. OLDSTONE§, M.D., and FRANK J. DIXON, M.D.

(From the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

(Received for publication 17 March 1970)

The New Zealand black (NZB), white (NZW), and their F1 hybrid (NZB × W) mice develop autoimmune phenomena which are fundamentally similar but differ in the time of onset, incidence, and severity from strain to strain and often, within the same strain, according to the sex. In the NZB strain the main manifestation of autoimmune disease is an autoimmune hemolytic anemia, although a significant proportion of mice also develop antinuclear antibodies (ANA) and glomerulonephritis (1). ANA and glomerulonephritis are most common in the NZB × W (2). The NZW mice, which were initially considered to be healthy, have been recently shown to also have antinuclear antibodies, glomerulonephritis, and positive Coombs test, but with a lower incidence and severity than in the other two strains (3). The etiology of the autoimmune phenomena in NZ mice remains unknown despite a large number of studies on this subject. According to some authors, murine leukemia viruses, which are naturally present in NZB mice, are responsible for the autoimmune disease at least in this strain (4). According to others, an immunologic hyperresponsiveness of the NZ mice to nucleic acid antigens and not a unique viral infection is the cause of autoimmune disease. In support of the second opinion, it has been shown that in some NZ mice at least two induced viral infections (5–7) or immunization with DNA (2) or synthetic polynucleotides (8) will enhance some of the manifestations of autoimmunity, the latter in spite of inducing significant levels of interferon.

The purpose of this study was to observe the effect of two different chronic viral infections, due to an RNA and a DNA virus, respectively, on the autoimmune disease of NZ mice. It was found that both viral infections induce an increased incidence of antinuclear antibodies and of mortality, presumably largely due to glomerulonephritis, in all NZ mice but not in other strains.

* This is publication No. 402 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California. This research was supported by United States Public Health Service Grant Al-07007 and Atomic Energy Commission Contract AT(04-3)-410.

‡ Supported in part by NATO Training Grant and at present by National Kidney Foundation Fellowship.

§ Recipient of Career Development Award 1 KO4 Al 42580-01 AlD from the United States Public Health Service.

Neither viral infection altered the incidence of anti–red cell antibodies or the severity of autoimmune hemolytic anemia.

Materials and Methods

Host.—Mice: Five lines of mice were investigated: NZB, NZW, NZB × W, SWR/J and B10D2 old line. Each group of experimental and control mice consisted of approximately 25 males and 25 females. NZB and NZW mice were originally obtained from the Laboratory Animal Center, Medical Research Council, Surrey, England, and inbred for 4 yr in our laboratory by brother-sister mating. The NZB female × NZW male cross and the reciprocal mating were both randomly performed. For comparative purposes SWR/J and B10D2 old line inbred mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and bred in our laboratory. Random testing of mice indicated that they were free of lymphocytic choriomeningitis (LCM) virus, lactic dehydrogenase virus (LDV), and polyoma virus infections.

Virus—LCM: The source of the virus employed and the methods of handling and diluting virus for inoculation have been previously described (7). Procedure for titration of infectious virus from tissues of carrier mice has been reported (7, 9).

Polyoma: Polyoma virus employed was mouse kidney passage small plaque forming type, kindly provided by Dr. K. Habel and derived from the seed originally obtained from Dr. Dulbecco (10). The virus was administered subcutaneously to newborn mice within the first 15 hr of life. The dose was .03 ml of a solution containing 1.5×10^7 p.f.u./ml. Titration of infectious virus in the tissues was performed according to the method of Dulbecco et al. (11). Initial 10% dilution of homogenized kidney from polyoma infected mice, cleared by centrifugation at 700 *g* for 10 min, was diluted 100-fold in EBM with 2% heat-inactivated fetal calf serum, and assayed for presence of infectious virus.

Clinical State.—At 3, 6, and 9 months blood was obtained from each mouse by orbital sinus puncture for serological and hematologic studies, and a 24 hr urine sample was collected.

Detection of antinuclear antibodies: ANA were detected by indirect immunofluorescent antibody technique as previously described (2). Titration was performed on all positive sera by serial twofold dilutions until end point was reached. The specificity of the antinuclear antibody was investigated by a modified Ouchterlony agar diffusion method (12) using as source of antibody either individual sera or pools of about 10 sera of mice of both sexes with high ANA titers and as antigens calf thymus native and heat-denatured DNA, soluble nucleoprotein (13, 14), and Sm antigen (12). For comparative purposes, human antisera specific for native DNA and soluble nucleoprotein and rabbit antiserum specific for denatured DNA were kindly provided by Dr. E. M. Tan.

Direct Coombs test: Rabbit anti–mouse gamma globulin serum was absorbed with an equal volume of 10 times washed normal mouse red cells to remove natural rabbit anti–mouse hemagglutinin. Test erythrocytes were washed three times in saline and a 2% cell suspension was incubated for 1 hr at room temperature with an equal volume of serial twofold (1:2–1:128) dilutions of the antiserum in microtitration plate wells. Known positive and negative controls were included in each determination.

Hematocrit: The hematocrit values were determined on freshly drawn blood by use of microhematocrit tubes.

Determination of proteinuria: Proteinuria was determined by the sulfosalicylic method (15) and reported as mg/24 hrs (2).

Histopathology.—At 9 months, or before if moribund, the mice were sacrificed for histologic study.

Immunofluorescence: Renal tissue blocks of each sacrificed mouse were snap-frozen in liquid nitrogen and sections 4 μ thick were fixed in acetone or ether-alcohol (7) and examined by the direct immunofluorescence technique using a rabbit fluorescein-labeled anti-mouse IgG. Additional kidney sections were also tested with rabbit anti-mouse albumin, anti-mouse C3, and anti-rat fibrinogen antisera. The latter cross-reacted strongly with mouse fibrinogen. Details as to preparation of antigens used and conjugation of $\gamma 2$ globulins from above antisera to fluorescein isothiocyanate have been reported (16, 17).

Histology: For all mice sacrificed, formalin-fixed paraffin sections of brain, thymus, heart, lung, liver, spleen, skeletal muscle, kidney, and omental fat were stained by hematoxylin and eosin and periodic-acid Schiff (PAS) methods. Several kidney sections were also stained with Congo red for amyloid detection.

RESULTS

New Zealand Mice

LCM Virus—Determination of viral infectivity titers of 6-wk-old LCM carrier NZ mice revealed in general viral concentrations intermediate between SWR/J and C_3H strains. At 6 wk of age, the NZB × W hybrids had higher tissue virus concentrations than the parent strains and the NZW had comparatively lower titers in the brain (Table I). A second observation of note is the uniform increase in virus titers in the NZ carriers between the 6th wk and 6th month, a change not usually seen in the non-NZ strains.

Polyoma Virus.—More infectious polyoma virus was detected in 9 month old NZB and NZB × W than NZW mice. Using a 10^{-3} dilution of kidney homogenate from infected NZ mice, only two of eight NZW mice had demonstrable polyoma virus, while five of six NZB and four of six NZB × W mice showed polyoma virus.

Clinical State.—

ANA: As shown in Table II, polyoma and to a greater extent LCM infection increased the incidence of ANA at all ages in all NZ mice. The most striking differences were observed at 3 and 6 months between the LCM infected animals and the corresponding controls in all NZ strains. None of the mice of the NZW LCM infected group were alive at 9 months, but the incidence of ANA had already reached values close to 100% by 6 months.

In NZB and NZW strains, the arithmetic mean ANA titer was increased at all ages by polyoma and LCM infections. In LCM infected NZB × W mice only 6-month females had titers lower than controls. In those infected with polyoma, 3-month females and 9-month males had titers lower than controls.

With NZB sera, the nuclear staining was almost invariably speckled or rim. With NZW and NZB × W the homogeneous, patchy and rim types of staining could be observed often in combination. In these two strains speckled staining was not seen.

By agar diffusion technique using pooled sera of usually 10 mice with high ANA titers, precipitating antibodies against heat-denatured DNA but not against native DNA or Sm antigen were detected in all three NZ lines, either

TABLE I

Concentration of Infectious Virus in Tissues of LCM Carrier Mice

Strain	Blood		Brain		Kidney	
	6 wk	6 months	6 wk	6 months	6 wk	6 months
NZB	2.1*	4.1	3.7	5.1	2.5	5.3
NZW	1.7	2.8	1.7	3.2	2.5	3.7
NZB×W	1.9	4.0	4.2	5.1	3.9	5.1
SWR/J	4.4	3.6	4.8	4.2	5.4	5.4
B10D2 old	2.9	1.8	3.1	−‡	3.6	3.4
C_3H	1.4	1.2	1.7	1.4	1.9	2.2

* Reciprocal of log dilution giving a 50% lethal end point per 0.03 ml intracerebral inoculum in Swiss Webster mice. Number is the mean value of LD_{50} end points obtained with organs from four individual mice.

‡ Titration not done.

TABLE II

% Cumulative Incidence and Titers of Antinuclear Antibody

	NZB						NZW						NZB × W					
	Control		Polyoma		LCM		Control		Polyoma		LCM		Control		Polyoma		LCM	
3 month																		
male	21*	<1‡	39	2	81	5	4	<1	22	2	64	10	0	<1	8	<1	46	17
female	8	<1	48	1	87	9	7	<1	33	5	64	10	13	9	40	5	63	23
6 month																		
male	21	<1	71	6	92	22	21	2	38	5	96	23	17	10	44	16	72	33
female	44	<1	65	4	86	5	38	3	47	8	94	118	50	52	96	74	100	43
9 month																		
male	45	<1	82	13	100	12	26	5	47	8	no		43	53	94	48	93	63
female,	53	<1	95	42	100	45	52	4	77	17	survivors		93	48	96	160	100	78

* % positive

‡ Number is the arithmetic mean of the reciprocal of the end point dilutions of all mice in group.

untreated or infected with either virus. In NZW mice sera precipitating antibodies against soluble nucleoprotein were also present (Table III).

Erythrocyte autoantibodies: The cumulative incidence of positive direct Coombs tests did not reveal consistent, significant differences between the control mice and those infected with either virus (Table IV). The highest values

were observed in the NZB mice where the average incidence for all groups exceeded 50% at 6 months and approached 100% at 9 months. In this strain the 6 and 9 month values were slightly higher in all groups of virus infected mice than in controls except for the LCM infected males at 6 months. In NZB × W

TABLE III

Detection of Precipitating Activity of Pools of Mice Sera by Immunodiffusion in Agarose

Antigen	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
N-DNA*	−	−	−	−	−	−	−	−	−
H-DNA‡	+	+	+	+	+	+	+	+	+
sNP§	−	−	−	+	+	+	−	−	−
Sm‖	−	−	−	−	−	−	−	−	−

* 1 mg/ml calf thymus DNA in phosphate buffered saline, pH 7.2.

‡ 500 μg/ml heat-denatured calf thymus DNA in phosphate buffered saline, pH 7.2.

§ Calf thymus nucleoprotein soluble in 0.015 M NaCl.

‖ Nuclear antigen extracted from calf thymus nuclei with phosphate buffered saline, pH 7.2.

TABLE IV

Cumulative % Incidence of Positive Direct Coombs Test

	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
3 month male	14	0	4	0	0	0	0	0	0
female	5	0	3	0	0	0	0	0	10
6 month male	50	56	43	0	0	0	0	0	9
female	53	75	75	0	0	0	16	21	23
9 month male	87	100	100	0	0	No sur-	32	11	62
female	86	100	100	0	7	vivors	82	50	71

hybrids the incidence of positive Coombs test at 6 months was also slightly higher in the infected mice than in controls but at 9 months the values were higher in controls than in three of the four infected groups. Among NZW the only positive Coombs tests were found in 7% of the 9 month old female polyoma infected mice.

Hematocrit: The mean hematocrit values (Table V) were similar in most control and virus infected groups of mice and in most instances were lower at

9 months than at 3 and 6 months. The most significant differences between control and infected mice were in the 9 month NZB's where the polyoma infected group had low values and the 6 month NZW's where those infected with LCM were also low. The 9-month NZB × W females, both control and infected, had low hematocrits, apparently reflecting the advanced renal disease in all these mice.

TABLE V

Mean Hematocrit Values

	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
3 month									
male	44	44	45	43	41	39	44	44	47
female	45	43	44	41	41	39	45	45	41
6 month									
male	42	42	42	43	39	37	44	43	43
female	43	42	42	44	42	37	42	41	45
9 month									
male	40	34	38	41	40	No sur-	41	38	46
female	40	36	39	42	39	vivors	33	36	36

TABLE VI

% Incidence of Significant Proteinuria in Female Mice*

	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
3 month	0	0	11	0	0	9	4	0	13
6 month	0	0	35	0	0	38	24	8	29
9 months	0	6	41	8	0	No sur-	63	56	90
						vivors			

* Higher than 5 mg/24 hr.

Proteinuria: The incidence of significant proteinuria (higher than 5 mg/24 hrs) is reported only for females (Table VI) since there is a high incidence of proteinuria in young males which is unassociated with renal disease, apparently related to testicular function (2). While the presence of proteinuria in female mice always indicates severe glomerular disease, the reverse is not necessarily true. A significant number of severely nephritic animals do not spill excessive amounts of protein, which no doubt accounts for the relatively low incidence of proteinuria seen in Table VI. In the NZB and NZW, strains which normally do

not develop significant proteinuria, LCM infection resulted in proteinuria in over a third of the animals by 6 months, reflecting the development of severe glomerulonephritis in most of these animals. On the other hand, whatever increment in renal disease is caused by polyoma infection does not appear to result in proteinuria. In the NZB × W mice the incidence of proteinuria in controls is high so that only a slight increment in LCM infected animals occurs.

Histopathology.—

Kidney: At gross examination, the most severely involved kidneys of all groups were enlarged, pale, and had numerous petechiae on their surface. Severe renal disease was often associated with anasarca.

TABLE VII

*% Incidence of Different Degrees of Glomerular Lesions**

	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
Negative/minimal‡	44	27	11	64	51	20	26	32	5
Moderate§	44	23	50	36	30	27	24	27	16
Severe‖	12	50	39	0	19	53	50	41	79
Total number of observations	16	26	18	44	43	30	37	22	19

* Degree of glomerular involvement as determined by histologic examination.

‡ Normal appearance or only focal mesangial deposition of PAS-positive material.

§ Glomerular hypercellularity, basement membrane thickening, Bowman's parietal cells proliferation in less than 70% of glomeruli.

‖ Same lesions as in moderate, present in more than 70% of glomeruli and very advanced in the single glomeruli.

At histologic examination a wide range of renal changes was observed (Table VII). The lesions were classified as minimal when only focal mesangial deposition of PAS-positive material was observed. The lesions were classified as moderate when less than 70% of glomeruli showed hypercellularity, either due to the presence of numerous polymorphonuclear neutrophils within the capillary lumens (Fig. 1) or to proliferation of mesangial and endothelial cells (Fig. 2), thickening of the basement membrane of glomerular capillaries (Fig. 2) and proliferation of the parietal cells of Bowman's capsule (Fig 3). The lesions were classified as severe when more than 70% of glomeruli showed these changes and in the single glomeruli they appeared very advanced. Some PAS-positive thrombi within the glomerular capillary lumens (Fig. 4) and tubular hyaline casts were often associated with the severe forms. In the most advanced lesions, many of the glomeruli appeared entirely transformed into a uniform mass of

eosinophilic material. Interstitial infiltrates of mixed inflammatory cells were observed in all groups of mice, more frequently associated with severe glomerular injury and/or viral infections. The renal lesions appeared to have similar histologic characteristics, with variations in intensity, in the three NZ strains

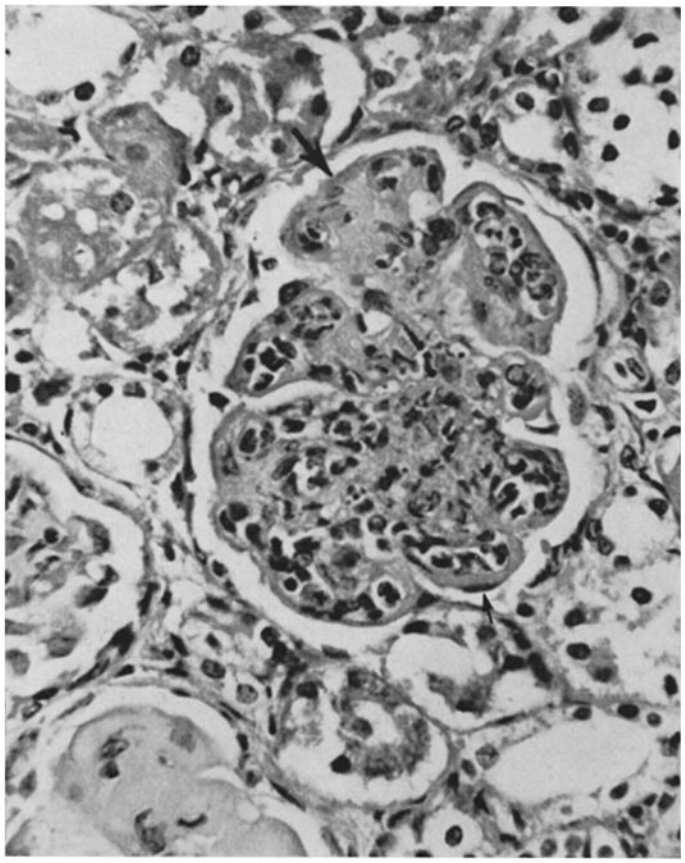

FIG. 1 Light photomicrograph of a hematoxylin- and eosin-stained kidney section from a 7 month old NZW LCM carrier mouse showing glomerular hypercellularity, largely due to the presence of numerous polymorphonuclear neutrophiles within the lumens of glomerular capillaries. Thickening of glomerular basement membrane (small arrow) and areas of fibrosis (large arrow) are also present. × 800.

either virus infected or controls. In all three strains the incidence of severe glomerular changes was higher in the LCM infected mice than in the controls. Such a difference was particularly evident in the NZW strain, in which about 50% of LCM infected mice and none of the controls had histologically severe glomerulonephritis. In polyoma infected mice, an increase in glomerular disease

could be observed in NZW and NZB strains but not in the NZB × W hybrids. In no instance was deposition of amyloid observed.

Other tissues: In untreated NZ mice the histology of liver, heart, and other nonlymphoid tissues appeared normal. The lymphoid tissues showed those

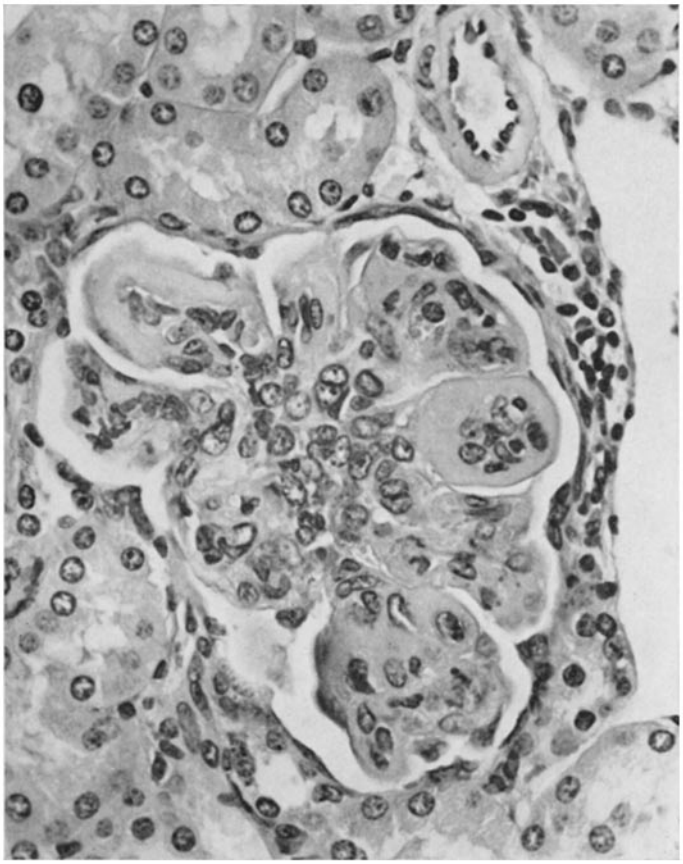

FIG. 2 Light photomicrograph of a PAS-stained kidney section from a 9 month old SWR/J polyoma carrier mouse showing severe thickening of the basement membrane of glomerular capillaries, the lumens of which are almost completely obliterated. The number of mesangial cells is increased. × 1200.

changes already described (1). In LCM infected mice, focal parenchymal necrosis and perivascular infiltrates of mixed inflammatory cells were often observed in liver and heart and less frequently in other body tissues (fat, skeletal muscle). The tissues of polyoma infected mice were comparable to those of controls except for a few instances of cellular infiltrates in the liver, much less

severe than in LCM infected mice. The incidence of tumors in polyoma infected animals appeared low in all three NZ lines, averaging 4%.

Immunofluorescence: The study of kidneys by direct immunofluorescent technique (using fluoresceinated rabbit anti-mouse IgG antiserum) revealed

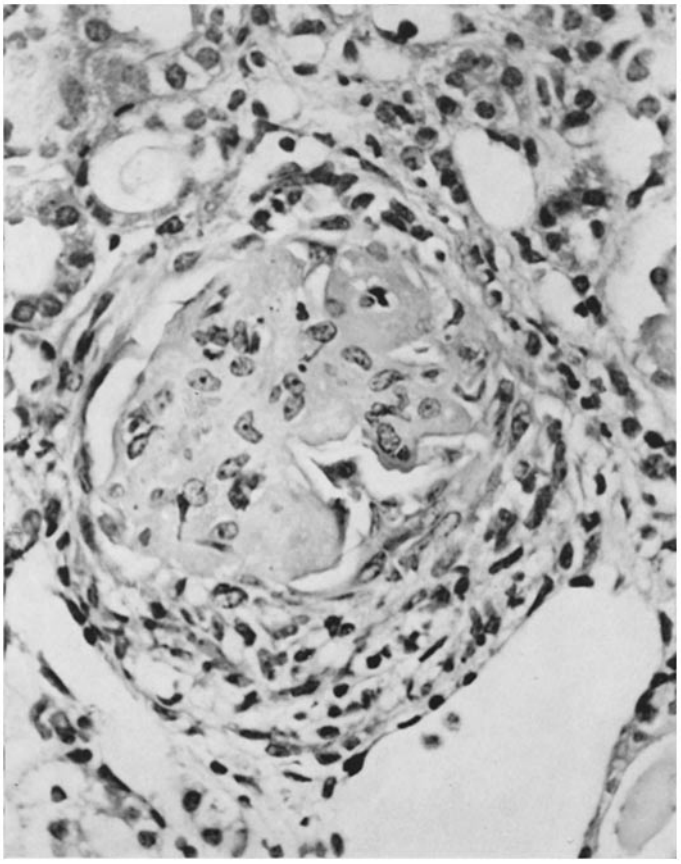

FIG. 3 Light photomicrograph of a PAS-stained kidney section of a 9 month old NZW LCM carrier mouse showing proliferation of the parietal cells of Bowman's capsule. The glomerulus is almost entirely transformed into a hyaline mass. × 1200.

IgG and C3 deposition in the glomeruli of infected animals and controls. The amount of deposited IgG ranged from minimal mesangial deposits to marked deposition in mesangia and peripheral capillary walls. The pattern of the deposits along the glomerular capillary walls was in almost all instances either finely or coarsely granular (Fig. 5). In 10 out of about 300 kidneys not correlated with strain or treatment, an apparently linear deposition of IgG along the

capillary walls was detected in association with the presence of mesangial deposits (Fig. 6). In NZW and NZB strains, the incidence of heavy IgG and C3 deposition was higher in LCM infected mice than in polyoma infected ones and in the latter was higher than in controls. No clearcut differences were

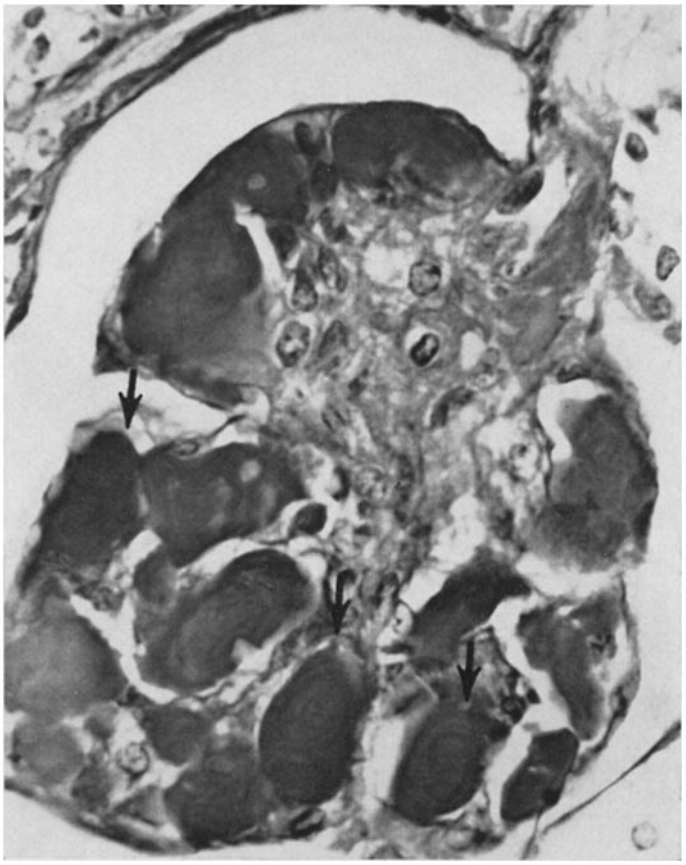

FIG. 4 Light photomicrograph of a PAS-stained kidney section of a 9 month old NZB × W LCM carrier mouse showing numerous intracapillary PAS-positive thrombi (arrows). × 2200.

observed between control and infected NZB × W hybrids. For each group of mice some of the kidneys with different degrees of IgG deposition were investigated for the presence of albumin, fibrinogen (Fig. 7), and C3. A close correspondence was observed between the amount of IgG and that of fibrinogen and C3 deposited in the glomeruli, whereas albumin did not appear to be specifically deposited.

In about 5% of observations of NZB × W hybrid kidneys by direct immunofluorescence for the detection of IgG the nuclei of epithelial tubular cells and even more so those of endothelial cells of intertubular vessels and glomeruli appeared brightly fluorescent, similar to a positive ANA staining as revealed

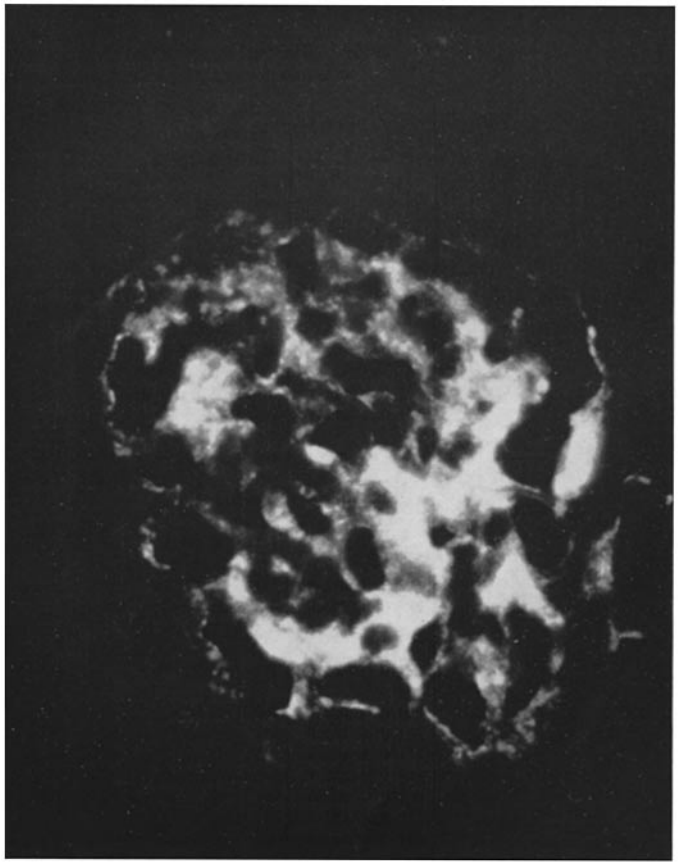

FIG. 5 Renal glomerulus from a 9 month old NZB polyoma carrier mouse. The preparation was stained with fluorescein-conjugated rabbit antiserum to mouse 7S γ globulin. One can observe the typical granular deposition of Ig in immune complex glomerulonephritis of mice. × 1200.

by indirect immunofluorescent technique (Fig. 8). Prolonged rinsing of the kidney sections with saline prior to staining with fluoresceinated antiserum did not reduce the intensity of nuclear fluorescence.

Mortality—The cumulative incidence of mortality (Table VIII) was highest in LCM infected NZ mice, next in polyoma infected, and lowest in the non-

infected controls. Such differences were evident by the third month in NZB and NZB × W mice and by the sixth month in the NZW strain and in most instances increased throughout the period of observation.

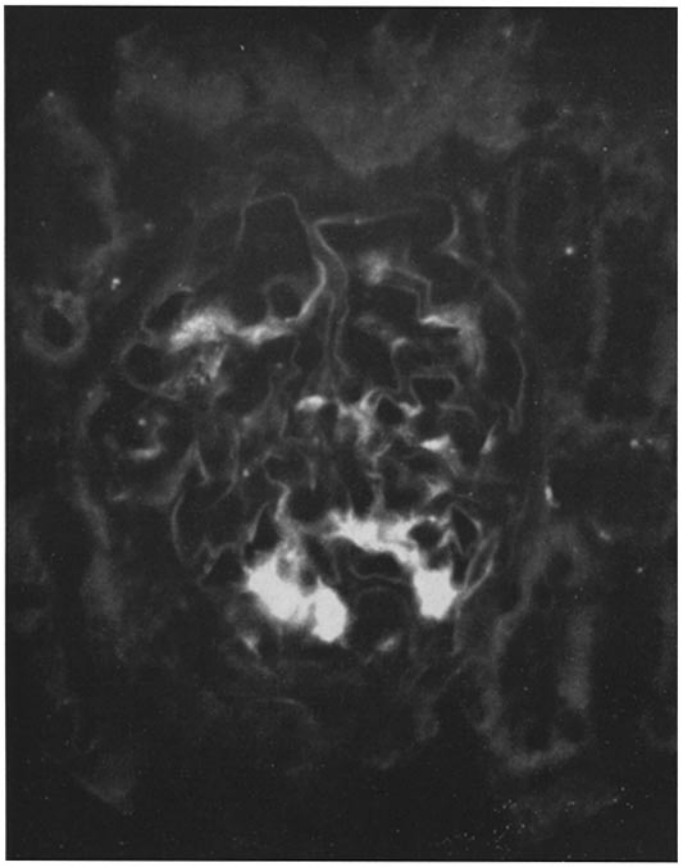

FIG. 6 Renal glomerulus from a 9 month old SWR/J polyoma carrier mouse. The preparation was stained with fluorescein-conjugated rabbit antiserum to mouse 7S γ globulin. γ globulin is deposited in mesangial areas in irregular masses and along peripheral walls of glomerular capillaries in an apparently linear pattern. × 900.

SWR/J and B10D2 Old Strains

Clinical State—LCM or polyoma chronic infections did not induce an increased incidence of ANA in these two strains. Only occasionally positive sera were found by 9 months in both control and virus infected groups. In no instances were positive Coombs test or anemia observed. Only in a few SWR/J LCM infected mice, proteinuria higher than normal was detected.

Histopathology.—The changes in kidney, liver, and other tissues of SWR/J and B10D2 old mice chronically infected with LCM virus have been already described in detail (7). The most pertinent changes in these mice were glomerulonephritis, focal hepatic necrosis, and interstitial lymphoid infiltrates seen in moderate degree in the SWR/J and to a lesser degree in B10D2 old.

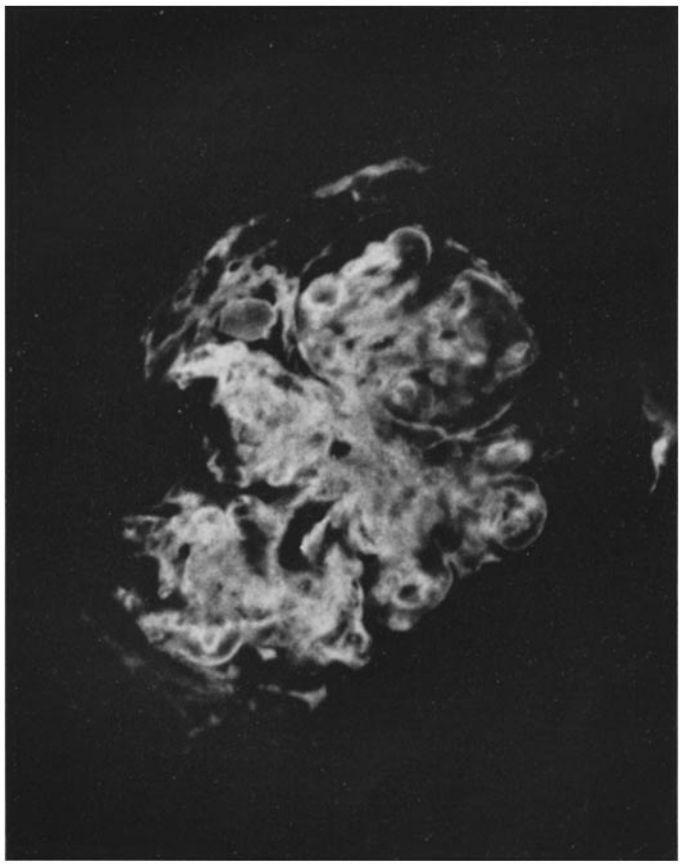

FIG. 7 Renal glomerulus from a noninfected 9 month old NZB × W mouse stained with fluorescein-conjugated rabbit anti-rat fibrinogen. The fibrinogen is present in a coarser pattern than the Ig, reflecting its heavy deposition within capillary lumens and mesangia as well as in Bowman's space. × 900.

In polyoma infected SWR/J's histologic study revealed in most kidneys an increased amount of PAS-positive material in mesangial areas, sometimes associated with the presence of mixed inflammatory infiltrates, and in about 10% more severe changes, thickening of the basement membrane, intracapillary hyalinization, and glomerular hypercellularity (Fig. 2). Extra renal tissues were essentially normal. Polyoma infected B10D2 old mice were histologically

normal. About 3% of polyoma infected SWR/J mice and none of polyoma infected B10D2 old mice had tumors.

Immunofluorescence.—The study of kidneys by direct immunofluorescence revealed, with a few exceptions, modest granular IgG and C3 deposition in the

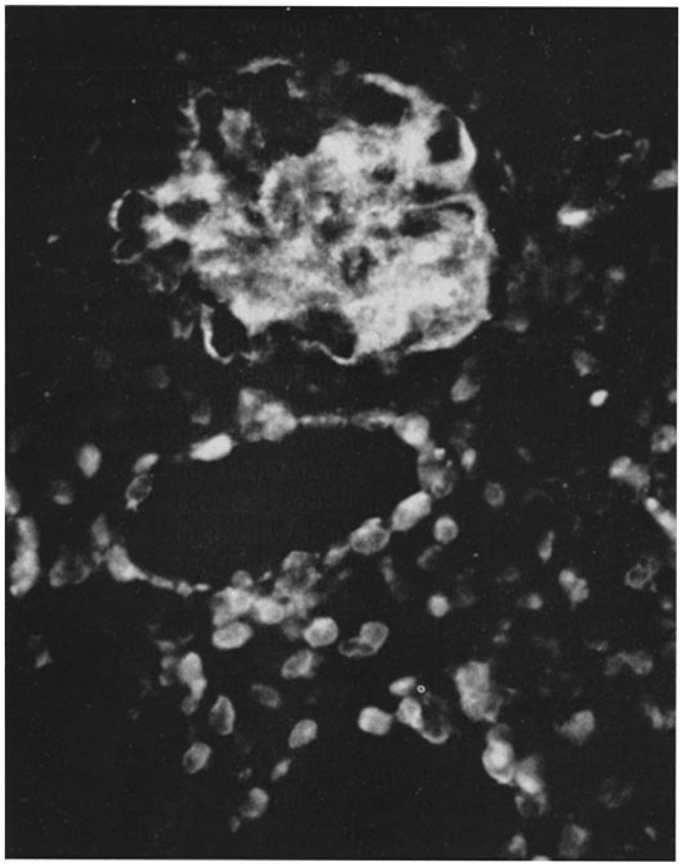

FIG. 8. Renal section from a 9 month old NZB × W polyoma carrier mouse. The preparation was stained with fluorescein-conjugated rabbit antiserum to mouse 7S γ globulin. γ globulin is deposited in mesangial areas and along peripheral walls of glomerular capillaries with a typical granular pattern. In addition, the nuclei of epithelial tubular cells and of endothelial cells of intertubular vessels are clearly stained. × 500.

glomeruli of SWR/J and B10D2 old mice infected with polyoma, while IgG and C3 in a heavier granular deposition were common in the glomeruli of both SWR/J and B10D2 LCM infected mice. In a single SWR/J mouse infected with polyoma, IgG was found in an apparently linear pattern along the capillary basement membranes.

Mortality.—In LCM infected SWR/J a 5% increase in the mortality compared with controls was observed by 9 months. In polyoma infected SWR/J and in either LCM or polyoma infected B10D2 old mice no increased mortality was detected.

TABLE VIII

% Cumulative Mortality

	NZB			NZW			NZB × W		
	Control	Polyoma	LCM	Control	Polyoma	LCM	Control	Polyoma	LCM
3 month									
male	0	0	38	8	0	0	0	0	32
female	0	4	40	0	0	0	0	0	38
6 month									
male	0	21	63	19	31	39	3	17	68
female	17	25	74	3	5	60	7	14	75
9 month									
male	21	42	88	27	59	100	13	42	97
female	22	43	82	13	23	100	67	71	92

DISCUSSION

The present observations indicate clearly that two quite dissimilar chronic viral infections caused by either LCM, an RNA virus, or polyoma, a DNA virus, enhance the ANA response, aggravate the immune complex glomerulonephritis, and increase the associated mortality of NZB, NZW and NZB × W mice but do not influence significantly the incidence of anti-erythrocyte antibodies or anemia. The manner in which these chronic viral infections increase ANA formation without apparently altering its immunologic specificity is not certain. The fact that either a DNA, or to an even greater extent an RNA virus, is effective would suggest that viral nucleic acid itself may not act as a specific antigen in this enhancement. A similar situation exists with purified nucleic acids, i.e., injection of either calf thymus heat-denatured DNA coupled to methylated BSA (2) or synthetic double stranded RNA (8) will increase the antinuclear or anti-DNA responses of NZ mice. In the case of polyoma infection or DNA injection there could be specific antigenic stimulation of ANA formation. However, in the case of LCM infection or RNA injection, if the RNA were acting as an antigen it would have to stimulate ANA formation as a related antigen causing a nonspecific secondary response (18). There are other possible explanations for this enhancement. The RNA, viral or synthetic, might act as a nonspecific adjuvant (19) increasing the response to nuclear antigens. That this is not likely is suggested by the reported immunosuppressive

effect of LCM infections (20, 21). Either induced infection might alter the course of a spontaneous infection already present in these mice thereby increasing the ANA response. Also, either viral infection, although generally considered to be noncytopathogenic, might increase the amount of host nuclear antigen made available to immunocompetent cells. Such an increase could be the result of either a minimal amount of cell injury with spilling of antigen and/or an interference with normal catabolism of the products of effete nuclei. The presence of abnormal amounts of DNA in the sera of NZB × W mice has been suggested (2).

There are at least two possible mechanisms by which the viral infections might increase the severity of the glomerulonephritis of the NZ mice. First, since both infections enhance the ANA response, and since nuclear antigen-ANA (or more specifically, DNA-anti-DNA) complexes are the suspected pathogens in the glomerulonephritis of NZB × W mice (2), they might exert some or all of their effect via this route. The nephritogenic effect of ANA could also be increased if the infections made available for the formation of circulating immune complexes larger amounts of host nuclear antigens as a result of cell injury. That increased amounts of nuclear antigen can rapidly accelerate the nephritic process was demonstrated by the i.v. injection of DNA into NZB × W mice with ANA (2). Second, since chronic viral infections can cause immune complex glomerulonephritis in most strains of mice via the formation of circulating viral-antiviral antibody complexes (7), this nephritogenic process could be added to the naturally occurring ANA induced nephritis of NZ mice. However, this is not likely to provide the entire explanation since the virus induced glomerulonephritis in most non-NZ strains develops much more slowly than the disease observed in the infected NZ mice. It would be particularly difficult to account for the severe nephritis in the LCM-infected NZW or NZB mice at 6 months on the basis of their spontaneous disease plus a viral nephritis of usual severity. It seems likely that both these mechanisms operate and their relative importance in the production of glomerular disease can be established only by determining the antibody specificity of the Ig found in the glomeruli, i.e., what proportion of the Ig reacts with nuclear antigens and what proportion with viral antigens.

The finding of immune complex glomerulonephritis associated with polyoma infection of both NZ and SWR/J mice adds it to the growing list of potentially nephritogenic viruses which now include: LCM (22, 23) and Moloney sarcoma (24) in mice and Aleutian virus in mink (25) where the anti-viral antibody has been eluted from the glomeruli, and LDV[1], Gross and Rauscher viruses[2], and Coxsackie virus (26) in mice and equine infectious anemia virus (27) in horses where infection is associated with immune complex glomerulonephritis.

[1] Oldstone, M. B. A., and D. D. Porter. Manuscript in preparation.

[2] Oldstone, M. B. A., and F. J. Dixon. Manuscript in preparation.

There are several aspects of the ANA and associated nephritis of NZ mice which warrant comment. The major ANA detected in all three NZ strains, infected or control, was directed against denatured DNA, and in the NZW strain antibodies to nucleoprotein were also found. By more sensitive labeled DNA binding techniques (28) antibodies to native DNA can also be found in some NZ mice but usually in amounts considerably less than the anti-denatured DNA antibodies[3]. There seemed to be little or no correlation between the nuclear staining pattern seen on the fluorescent antibody test and the specificities of the antibody detected in Ouchterlony reactions; however, these reactions may well have missed antibodies involved in determining patterns of ANA staining. The finding of host Ig apparently fixed to nuclei in vivo in 15% of NZB × W mice corresponds to similar observations reported in humans with systemic lupus (29). If this is not artifact, which seems unlikely, it indicates that a reaction between host Ig and nuclei of apparently intact cells had taken place in vivo. Whether an abnormal permeability of cell surfaces or an unusual Ig molecule might be involved is not known. The finding of deposits of fibrin in the more severely involved glomeruli implies the rapidity of the nephritogenic process. The deposition of fibrin especially in Bowman's space usually accompanies explosive immunologic inflammation and indicates irreversible glomerular injury and scarring.

The three types of NZ mice appear to share some unusual immunologic responses, but they differ considerably in their handling of chronic viral infections. While these mice are not unique in their spontaneous formation of ANA, the amount of this antibody formed by NZB × W and NZB mice exceeds that found in other strains. They also suffer much more from the immune complex glomerulonephritis associated with ANA than do other mice. In addition, all NZ mice responded to LCM and polyoma infections with enhanced ANA titers within the first 3 months of life. Such a response is not seen during the first 9 months of life in the SWR/J or B10D2 old controls of this study nor in B10D2 new, AKR, or C_3H strains observed in other experiments. On the other hand, the three NZ mice handle chronic viruses quite differently. Analysis of sera of 2–3 month old NZB × W, NZB, and NZW mice for Gross antigen revealed its presence in the NZB × W and NZB but not in NZW[4]. Similarly, the NZW mouse carried less LCM and polyoma virus than the other strains after neonatal infection. The basis for the lower viral titers in NZW mice is not known but their lesser infection correlates with less severe "autoimmune" disease. While NZB mice were hyperresponsive to the Gross agent and actually eliminated it from the blood after 1 yr of age, all three types of NZ mice showed increasing LCM titers from 6 wk to 6 months of age, a time during which other strains showed no increase in LCM titers.

[3] McConahey, P. J. Unpublished observations.

[4] Aoki, T. Personal Communication.

The basis of the immunologic diseases of NZ mice is still not clear but it seems that both host and environmental factors may play a role. The ANA and associated glomerulonephritis of these mice appear to be predestined by their hyperreactivity to nuclear antigens, particularly DNA, plus any deficiencies they may have in degrading nuclear antigens. However, a predisposition to immunologic hyperreactivity, presumably on a genetic basis, is not in itself sufficient to cause disease as is evidenced by the relatively healthy NZW mice that can respond strongly to DNA (6). Superimposed infection (5), or other similar events, may be needed to activate this latent responsiveness by one or more of the mechanisms discussed above. The naturally occurring Gross infection in NZB × W[4] and NZB (4) mice might provide such a stimulus and its relatively low level or absence in NZW mice[4] would fit with their comparative well being. However, other, as yet unidentified, infections may also be involved in activating the autoimmune potential of these mice. The reports of polyoma and GD VII virus in some NZ colonies (4) emphasize the importance of this possibility. If the Gross agent is involved in the pathogenesis of NZ ANA and nephritis it is most likely to operate in a manner similar to that of LCM and polyoma, i.e., a nonspecific stimulation of native hyperresponsiveness, and not in a more specific manner (4). The wide distribution of the Gross agent among murine strains without associated ANA even in the presence of induced LCM or polyoma infections fits with this view and emphasizes the role of host factors in the immunologic diseases of NZ mice. In view of quite independent responses of red cell antibodies and ANA to LCM and polyoma infections it seems doubtful whether these two aberrant antibody responses are initiated by a single mechanism such as Gross infection (4).

SUMMARY

Chronic infections induced at birth with either LCM, an RNA virus, or polyoma, a DNA virus, in NZB, NZW, and NZB × W mice enhance ANA formation, aggravate the immune complex glomerulonephritis, and increase the associated mortality. The ANA titer was increased without apparent change in specificity of the antibodies involved in all three types of mice. Glomerulonephritis, while more severe in infected mice, was of the same type as occurred spontaneously and was characterized by a granular to lumpy accumulation of host IgG and C3 in the mesangia and along the capillary walls of the glomeruli. Of the LCM infected mice of all three types over 50% had died of glomerulonephritis by 6 months and over 85% by 9 months. Of the polyoma infected mice of all three types approximately 20% had died of glomerulonephritis by 6 months and over 40% by 9 months. Of the uninfected controls of all three types less than 10% had died by 6 months and less than 20% at 9 months except for the NZB × W females which had a 67% mortality at 9 months as a result of their spontaneous glomerulonephritis.

The two viral infections had no significant effect on the incidence of anti–red cell antibodies or the severity of autoimmune hemolytic anemia in any of the three NZ mice.

We thank Mrs. Susan Carter, Miss Antoinette Tishon, and Mr. Gerry Sandford for their capable technical assistance.

BIBLIOGRAPHY

1. Howie, J. B., and B. J. Heyler. 1968. The immunology and pathology of NZB mice. *Advan. Immunol.* **9**:215.
2. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**:507.
3. Hahn, B. H., and L. E. Shulman. 1969. Autoantibodies and nephritis in the white strain (NZW) of New Zealand mice. *Arthritis Rheum.* **12**:355.
4. Mellors, R. C., T. Aoki, and R. J. Huebner. 1969. Further implication of murine leukemia-like virus in the disorders of NZB mice. *J. Exp. Med.* **129**:1045.
5. Dixon, F. J., M. B. A. Oldstone, and G. Tonietti. 1969. Virus induced complex type glomerulonephritis. *Transplant. Proc.* **1**:945.
6. Lambert, P. H., and F. J. Dixon. 1970. Genesis of antinuclear antibody in NZB/W mice. Role of genetic factors and of viral infections. *Clin. Exp. Immunol.* In press.
7. Oldstone, M. B. A., and F. J. Dixon. 1969. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. *J. Exp. Med.* **129**:483.
8. Steinberg, A. D., S. Baron, and N. Talal. 1969. The pathogenesis of autoimmunity in New Zealand mice. I. Induction of antinucleic acid antibodies by polyinosinic polycytidylic acid. *Proc. Nat. Acad. Sci. U.S.A.* **63**:1102.
9. Oldstone, M. B. A., and F. J. Dixon. 1968. Susceptibility of different mouse strains to lymphocytic choriomeningitis virus. *J. Immunol.* **100**:355.
10. Vogt, M., and R. Dulbecco. 1962. Studies on cells rendered neoplastic by polyoma virus: the problem of the presence of virus-related materials. *Virology.* **16**:41.
11. Dulbecco, R., and G. Frieman. 1959. Plaque production by the polyoma virus. *Virology.* **8**:396.
12. Tan, E. M., and H. G. Kunkel. 1966. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **90**:464.
13. Tan, E. M. 1967. An immunologic precipitin system between soluble nucleoprotein and serum antibody in systemic lupus erythematosus. *J. Clin. Invest.* **46**:735.
14. Tan, E. M., P. H. Schur, R. I. Carr, and H. G. Kunkel. 1966. Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. Clin. Invest.* **45**:1732.
15. Kingsbury, F., C. Clark, G. Williams, and A. Post. 1926. Rapid determination of albumin in urine. *J. Lab. Clin. Med.* **11**:981.
16. Oldstone, M. B. A., and F. J. Dixon. 1968. Immunohistochemical study of allergic encephalomyelitis. *Amer. J. Path.* **52**:251.

17. Unanue, E., and F. Dixon. 1964. Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. *J. Exp. Med.* **119:**965.
18. Dixon, F. J., and P. H. Maurer. 1955. Specificity of the secondary response to protein antigens. *J. Immunol.* **74:**418.
19. Johnson, A. G., J. Schmidtke, K. Merritt, and I. Han. 1968. Enhancement of antibody formation by nucleic acids and their derivatives. *In* Nucleic Acids in Immunology. O. J. Plescia and W. Braun, editor. Springer-Verlag, New York. 379.
20. Mims, C. A., and S. Wainwright. 1969. The immunodepressive action of lymphocytic choriomeningitis virus in mice. *J. Immunol.* **101:**717.
21. Oldstone, M. B. A., and A. Tishon. 1970. The immune response of mice persistently infected with lymphochoriomeningitis virus to non-viral antigens. *Fed. Proc.* **29:**435.
22. Oldstone, M. B. A., and F. J. Dixon. 1967. Lymphocytic choriomeningitis: production of antibody by "tolerant" infected mice. *Science* (*Washington*). **158:** 1193.
23. Oldstone, M. B. A., and F. J. Dixon. 1969. Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. *J. Exp. Med.* **129:**483.
24. Hirsch, M. S., A. C. Allison, and J. J. Harvey. 1969. Immune complexes in mice infected neonatally with Moloney leukaemogenic and murine sarcoma viruses. *Nature* (*London*). **223:**739.
25. Porter, D. D., A. E. Larsen, and H. G. Porter. 1969. The pathogenesis of Aleutian disease of mink. I. In vivo viral replication and the host antibody response to viral antigen. *J. Exp. Med.* **130:**575.
26. Sun, S., G. E. Burch, R. S. Sohal, and K. Chu. 1967. Coxsackie B_4 viral nephritis in mice and its autoimmune-like phenomena. *Proc. Soc. Exp. Biol. Med.* **126:**882.
27. Banks, K. L., and J. B. Henson. 1969. Glomerular deposition of gamma globulin and complement (C'3) in equine infectious anemia. *Fed. Proc.* **28:**752.
28. Wold, R. T., F. E. Young, E. M. Tan, and R. S. Farr. 1968. Deoxyribonucleic acid antibody: a method to detect its primary interaction with deoxyribonucleic acid. *Science* (*Washington*). **161:**806.
29. Hench, P. K., C. B. Wilson, and E. M. Tan. 1969. In vivo fixation of gamma globulin in renal cell nuclei of patients with systemic lupus erythematosus (SLE). American Rheumatism Association, Interim Scientific Session, Tucson, Arizona. (Abstract).