

Safety Assessment of Poly I:C in NZB/NZW Mice (38565)

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Polyribinosinic:polyribocytidylic acid (poly I:C) has been shown to be a highly active inducer of interferon that is capable of preventing or limiting viral infections *in vivo* and *in vitro* (1-3). It has also been found to be a nonspecific stimulator of the immune system (4). The findings have been of sufficient promise to justify pursuit of application of the drug to prevention and treatment of viral and other diseases of man and domestic animals.

Extensive tests for acute toxicity in experimental animals established dosage levels for limited trials of the drug given intravenously in human subjects (5-7). Patients with advanced cancer were given repeated intravenous doses of poly I:C up to 4000 $\mu\text{g}/\text{kg}$ for therapeutic purpose (2, 8). None showed apparent adverse effect other than temperature elevation of short duration. The subjects did not develop detectable antibodies against poly I:C or against native or denatured DNA.

Steinberg *et al.* (9) reported tests of poly I:C in NZB/NZW mice that develop spontaneous and progressive disease resembling systemic lupus of man (10, 11). Young female mice given multiple intraperitoneal injections of poly I:C (3 $\mu\text{g}/\text{g}$ body wt) showed increased severity of glomerular lesions and increased incidence of RNA and DNA antibodies compared with appropriate control animals.

NZB/NZW mice are immunologically hyperreactive and hence, the results of tests with poly I:C in these animals may give spurious predictions as to what the drug might do in man. Nevertheless, studies to measure prophylaxis in normal human subjects demand consideration of the findings and the development of regimens for poly I:C administration that would not evoke the demonstrated undesirable effects in NZB/NZW mice. The findings in the present studies confirm the work of Stein-

berg *et al.* (9) following intraperitoneal administration of large doses of the drug, and show that the drug elicits only minimal adverse effect when given in limited dosage by the intranasal route.

Methods. Animals. Colonies of inbred NZB and NZW mice were maintained in the Merck Sharp and Dohme Research Laboratories. The NZB/NZW F₁ progeny were obtained by mating NZB male with NZW female mice. The ICR/Ha inbred mice have been maintained in these laboratories for many years. Animals in the experimental treated and control groups were randomized with respect to age and litter origin in order to minimize the considerable variation in onset and degree of spontaneous glomerular lesions that occur in NZB/NZW mice.

Poly I:C. Poly I:C was prepared in these laboratories by methods recorded previously (1). The poly I ($S_{20,w} = 14$) was complexed with poly C ($S_{20,w} = 6$) in pyrogen-free phosphate-buffered saline solution. The complex had a T_m of 62° and 37% hyperchromicity on melting. Phosphate buffered saline solution was used for placebo control purpose.

Treatment regimens. Drug concentration for each treatment group was determined weekly based on the average body weight of the animals in each group. Intranasal and intraperitoneal administrations were in 0.1 ml vol and were given under ether anesthesia.

Treatment groups. Groups 1a and 1c (Table I). Poly I:C (3 $\mu\text{g}/\text{g}$ per dose) or placebo was given intraperitoneally to 5- to 13-wk-old NZB/NZW (1a) and to 10-wk-old ICR/Ha (1c) mice three times per week during a period of 14 wk (40 doses). **Group 1b.** Another group of NZB/NZW mice, 5-6 wk of age, were treated as in group 1a, but for a period of 18 wk (52 doses). The animals in groups 1a, 1b, and

TABLE I. EFFECT OF INTRAPERITONEAL TREATMENT WITH POLY I:C (3 µg/g—3x/wk).

Strain of mice	Regimen	Total number of doses	Age (weeks) at sacrifice	Rate (No. positive/Total no. of animals)			
				Nucleic acid antibody		Glomerular lesions	
				Poly I:C	Δ DNA ^a	Individual animals	Average extent of lesions
NZB/NZW (Group 1a)	<u>14-wk study</u>						
	<u>Females</u>						
	Poly I:C treated	40	20–28	4/14 (29%)	12/14 ^b (86%)	14/14 ^c (100%)	2.5
	Placebo control	40	20–28	1/16 (6%)	8/16 (50%)	6/16 (38%)	1.5
	<u>Males</u>						
	Poly I:C treated	40	20–28	2/13 (15%)	12/13 ^d (92%)	4/13 ^b (31%)	1
NZB/NZW (Group 1b)	<u>18-wk study</u>						
	<u>Females</u>						
	Poly I:C treated	52	24–25	5/9 ^d (56%)	6/9 (67%)	9/9 ^c (100%)	3
	Placebo control	52	24–25	1/15 (7%)	8/15 (53%)	2/15 (13%)	1
	<u>Males</u>						
	Poly I:C treated	52	24–25	2/11 (18%)	4/11 (36%)	4/11 (36%)	1.5
ICR/Ha (Group 1c)	<u>14-wk study</u>						
	<u>Females</u>						
	Poly I:C treated	40	25	4/25 (16%)	1/25 (4%)	0/25 (0%)	0
	Placebo control	40	25	0/25 (0%)	0/25 (0%)	0/25 (0%)	0
	<u>Males</u>						
	Poly I:C treated	40	25	1/17 (6%)	0/17 (0%)	0/17 (0%)	0
	Placebo control	40	25	0/20 (0%)	0/20 (0%)	0/20 (0%)	0

^a Denatured DNA.^b 0.05 < P < 0.10.^c P < 0.001.^d P < 0.05.

1c were sacrificed 5–8 days after the last treatment. *Group 2a* (Table II). Poly I:C (0.2 µg/g per dose) or placebo was given intranasally to 5- to 13-wk-old NZB/NZW mice and to 10-wk-old ICR/Ha mice three times per week during a 14-wk period (40 doses), and were sacrificed at ages 20–28 wk and 25 wk, respectively, 5–8 days after the last treatment. Untreated NZB/NZW and ICR/Ha mice at 21–22 wk and 32 to 36 wk of age were selected for comparative study. *Group 3a* (Table III). Poly I:C (1 µg/g per dose) or placebo was given intranasally to NZB/NZW and to ICR/Ha mice 15–22 wk of age once weekly for 6 doses and sacrificed 1 wk after the last dose, at 21–28 wk of age. *Group 3b*. Another group of NZB/NZW mice were treated as in group 3a starting at 13 wk of age and sacrificed at 26 wk of age after an additional 8 wk without treatment following the sixth weekly dose. *Group 3c*. Poly I:C (1 µg/g

per dose) was given intraperitoneally to 19- to 20-wk-old NZB/NZW mice once weekly for six doses and sacrificed 1–1.5 wk following the last dose, at 26 wk of age. Control animals in each treatment group received placebo in the same regimen. All animals were bled for serologic studies and sacrificed at the ages stated in the tables. All sera were stored frozen at –20° until tested for presence of antibodies against poly I:C and heat denatured DNA by the complement-fixation (CF) procedure described earlier (12, 13). Positive sera were determined as having a CF titer of 1:10 or greater as defined by the greatest dilution of serum that gave 50% fixation of complement. Assays for antibody against native DNA by complement fixation gave results that showed no correlation with autoimmune disease and the findings were discarded.

Histology. The kidneys removed from the animals at autopsy were fixed with

TABLE II. EFFECT OF INTRANASAL TREATMENT WITH POLY I:C (0.2 µg/g—3x/wk).*

Strain of mice	Regimen			Rate (No. positive/Total no. of animals)			
	Treatment group	Total number of doses	Age (weeks) at sacrifice	Nucleic acid antibody		Glomerular lesions	
				Poly I:C	Δ DNA ^a	Individual animals	Average extent of lesions
NZB/NZW	14-wk study						
	Females						
	Poly I:C treated	40	20–28	6/17 ^b (35%)	11/17 (65%)	8/17 (47%)	1.5
	Placebo control	40	20–28	1/15 (7%)	7/15 (47%)	7/15 (47%)	1.5
	Untreated control	—	21–22	2/23 (9%)	10/23 (43%)	17/25 (68%)	1
(Group 2a)	Untreated control	—	32–36	3/10 (30%)	6/10 (60%)	10/10 (100%)	2.5
	Males						
	Poly I:C treated	40	20–28	0/9 (0%)	1/9 (11%)	0/9 (0%)	0
	Placebo control	40	20–28	0/11 (0%)	5/11 (45%)	0/11 (0%)	0
	Untreated control	—	21–22	1/25 (4%)	3/25 (12%)	6/25 (24%)	1

* No glomerular lesions or antinuclear antibodies were detected among 140 ICR/Ha mice included in this study: 21 males and 25 females were treated; 18 males and 22 females were placebo controls; and 27 males and 27 females were untreated controls.

^a Denatured DNA.

^b 0.05 < *P* < 0.10.

TABLE III. EFFECT OF INTRANASAL AND INTRAPERITONEAL TREATMENT WITH POLY I:C (1 µg/g—1x/wk).*

Strain of mice	Regimen			Rate (No. positive/Total no. of animals)			
	Treatment group	Total number of doses	Age (weeks) at sacrifice	Nucleic acid antibody		Glomerular lesions	
				Poly I:C	Δ DNA ^a	Individual animals	Average extent of lesions
NZB/NZW	6-wk intranasal study						
	Females						
	Poly I:C treated	6	21–28	1/39 (3%)	4/39 (10%)	38/40 (95%)	1.5
	Placebo control	6	21–28	6/41 (15%)	7/41 (17%)	41/41 (100%)	1.5
(Group 3a)	Males						
	Poly I:C treated	6	21–28	1/44 (2%)	1/44 (2%)	9/45 (20%)	1
	Placebo control	6	21–28	0/43 (0%)	0/43 (0%)	16/44 (36%)	1
NZB/NZW	14-wk intranasal study						
	Females						
	Poly I:C treated	6	26	1/10 (10%)	6/10 ^b (60%)	10/10 (100%)	2
	Placebo control	6	26	0/11 (0%)	1/11 (9%)	12/12 (100%)	2
(Group 3b)	Males						
	Poly I:C treated	6	26	1/12 (8%)	3/12 (25%)	7/12 (58%)	1
	Placebo control	6	26	1/11 (9%)	1/11 (9%)	4/11 (36%)	1
NZB/NZW	6-wk intraperitoneal study						
	Females						
	Poly I:C treated	6	26	21/29 (72%)	15/29 (52%)	30/30 ^c (100%)	2
	Placebo control	6	26	16/28 (57%)	17/28 (61%)	22/29 (76%)	1.5
(Group 3c)	Males						
	Poly I:C treated	6	26	11/26 (42%)	3/26 ^d (12%)	10/26 (38%)	1
	Placebo control	6	26	6/25 (24%)	9/25 (36%)	8/26 (31%)	1

* No glomerular lesions or antinuclear antibodies were detected among 93 ICR/Ha mice included in the 6-wk intranasal study: 22 males and 26 females were treated; and 19 males and 26 females served as placebo controls.

^a Denatured DNA.

^b *P* < 0.05.

^c *P* < 0.01.

^d 0.05 < *P* < 0.10.

formalin and embedded in paraffin by standard procedures. Transverse sections (5 µ thick) were cut across the cortex and through the papillae. Staining was by hematoxylin and eosin, alcian blue-PAS or

chromotrope silver methanamine. All sections were examined by one of us (B. M.) without knowledge of the sex or treatment of the animals.

Results. Histopathological classification of

renal glomerular lesions. Spontaneously occurring lesions in NZB/NZW mice were characterized mainly by proliferation of the endothelial and mesangial cells and by deposition of hyaline material along the capillaries and in the mesangium. In this study, the changes were recorded on a scale from 4 for markedly affected kidneys to 0 for normal kidneys. Kidneys scored as 4 showed glomerular changes approaching sclerosis and frequently revealed interstitial fibrosis, tubular atrophy and hypertrophy or necrosis of the glomerular arterioles. Kidneys were scored as 3 when vascular tufts in most glomeruli were affected and enlarged to twice-normal size (Fig. 1). Kidneys scored as 2 showed slight enlargement of a small proportion of the glomeruli. A score of 1 was given when detectable alterations occurred in a few of the glomeruli.

The spontaneous occurrence of glomerular lesions in untreated NZB/NZW mice is illustrated in Table II. By 21–22 wk of age, 17 of 25 NZB/NZW female mice and six of 25 NZB/NZW male mice had glomerular lesions and these were of roughly equal degree. There was a greater frequency of antibodies against heat denatured DNA than against poly I:C. The higher rate for development of autoimmune disease manifestations in female than in male NZB/NZW mice has been observed previously (10, 11). By 32–36 wk of age, all of 10 female mice showed glomerular involvement and there was more frequent presence of antibody against denatured DNA and poly I:C.

ICR/Ha mice did not develop glomerular lesions (Fig. 2). Scattered mononuclear cell and plasma cell infiltration was observed around the renal blood vessels and pelvis, and in the interstitial tissue of untreated as well as treated mice. This was observed also in NZB/NZW mice.

Intraperitoneal treatment: 3 μg poly I:C/g, three times per wk. Table I presents the data obtained with NZB/NZW or ICR/Ha mice given the drug for 14 or 18 wk (Groups 1a, 1b, 1c). The treated female NZB/NZW mice (groups 1a and 1b) showed a marked increase in incidence and in degree (scores 2.5 and 3) of glomerular lesions compared with the placebo controls

(scores 1.5 and 1.0). Male NZB/NZW (groups 1a and 1b) animals showed far less frequent and lower grade lesions, but were significantly affected by treatment. There was greater frequency of antibodies against poly I:C and against denatured DNA in both the treated female and male mice than in the controls. ICR/Ha mice (group 1c) did not develop glomerular lesions and only a few of the treated mice developed antibodies against poly I:C. A single treated animal showed antibody against denatured DNA.

Intranasal treatment: 0.2 $\mu\text{g}/\text{g}$, three times per wk. As shown in Table II, 40 doses of poly I:C given to NZB/NZW mice over 14 wk with sacrifice at 20–28 wk of age did not increase the percentage of female or male animals with glomerular lesions compared with the controls nor did the drug increase the degree of the lesions when they were present (group 2a). Furthermore, the drug did not significantly affect the incidence of animals with detectable antibodies to poly I:C or denatured DNA. The results of the tests with heat denatured DNA were not consistent and there was no apparent stimulatory effect of the drug. ICR/Ha mice treated in a similar way did not develop glomerular lesions or antibody against nucleic acids.

Intranasal or intraperitoneal treatment: 1 $\mu\text{g}/\text{g}$, one time per wk. Table III summarizes the results of treating NZB/NZW mice with six doses of poly I:C by the intranasal or intraperitoneal route. The groups of mice represented in studies 3a, 3b, and 3c were initiated at different time periods and have no direct relationship to each other. The animals given poly I:C in study 3a, in which sacrifice was within days following the sixth dose, showed no apparent potentiation either of the extent of the glomerular lesions or of the development of antibody against nucleic acids. Study 3b was similar to 3a with respect to treatment regimen except that the animals were all held to 26 wk of age; the last 8 wk without treatment. There was no great potentiation of glomerular lesions but there was increase in induction of antibody to denatured DNA. There was little change with regard to the antibody responses against poly I:C. Poly I:C given

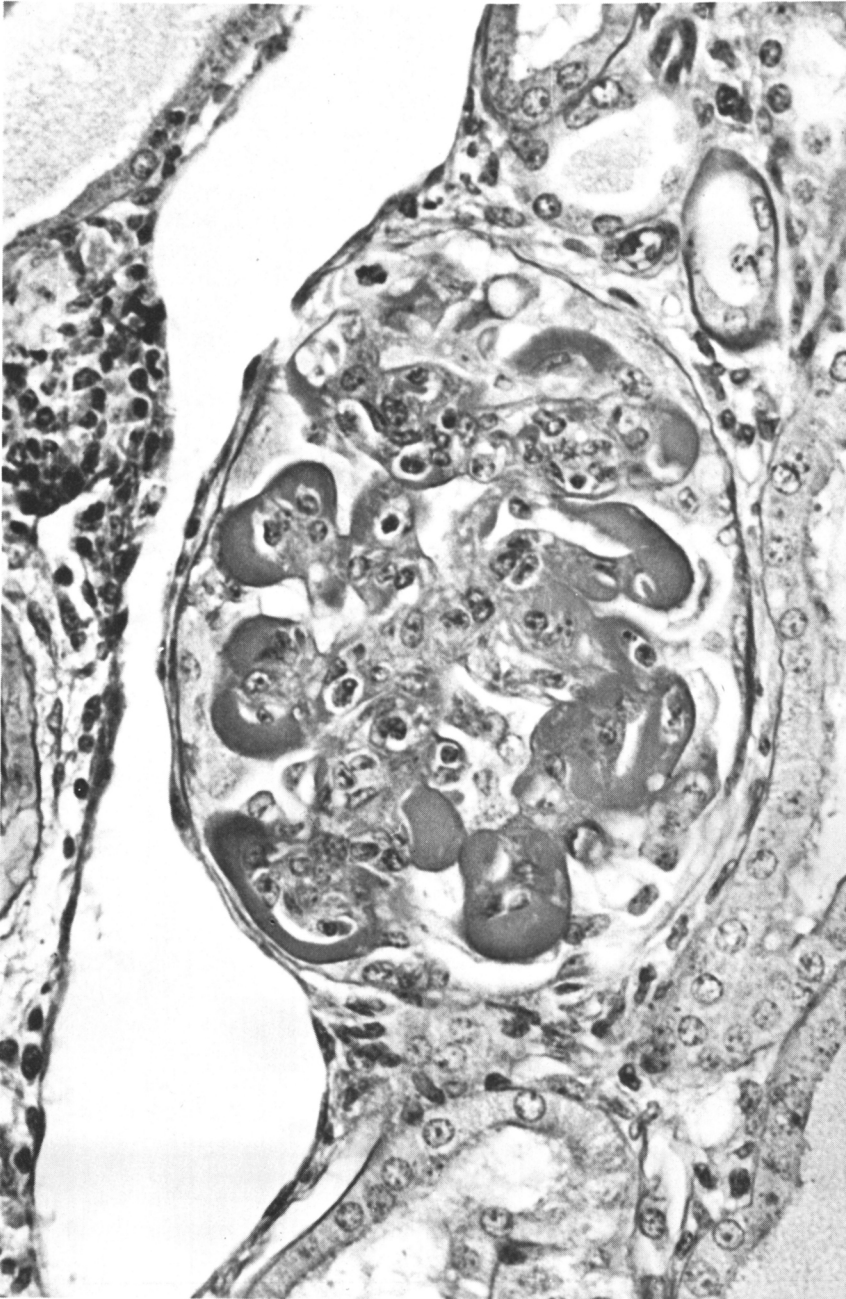


FIG. 1. Lupus-like glomerular lesions in NZB/NZW mouse, graded as moderate in degree. Stained with Alcian blue-PAS and magnified approximately 370X.

in six doses by the intraperitoneal route (group 3c) appeared to have little effect on the generation of glomerular lesions or of nucleic acid antibody in the animals. None of the ICR/Ha mice treated intranasally

showed potentiation of antinucleic acid antibodies or of glomerular lesions.

Discussion. The present findings confirm the work of Steinberg *et al.* (9) that NZB/NZW mice given poly I:C by the intra-

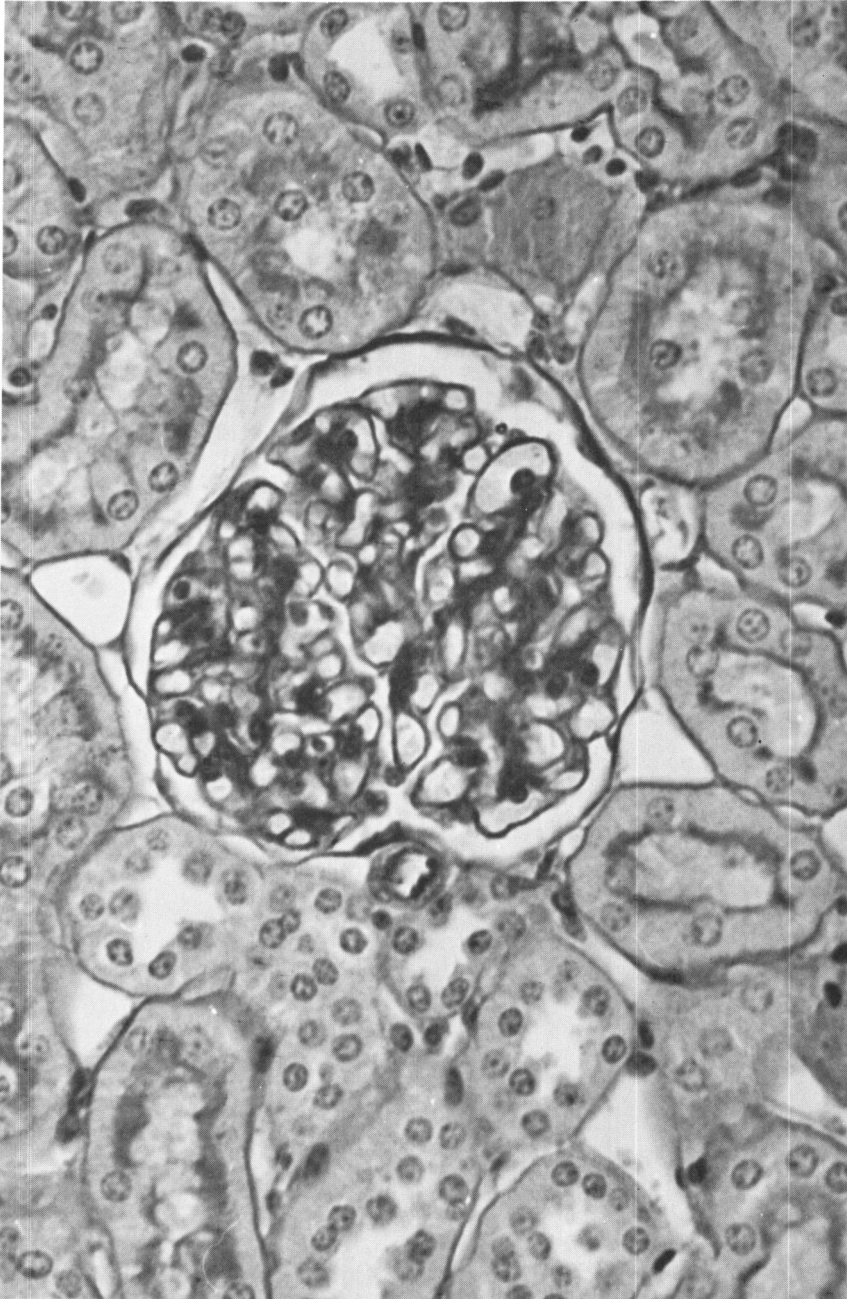


FIG. 2. Normal glomerulus in ICR/Ha mouse. Stained with Alcian blue-PAS and magnified approximately 370X.

peritoneal route over long periods of time show potentiation of their spontaneous autoimmune disease, as expressed here in terms of higher incidence and degree of glomerular lesions and antibody against

poly I:C and heat denatured DNA. The spontaneous disease was most prominent in female animals and potentiation of the disease by poly I:C was also most prominent in animals of this sex.

Intraperitoneal administration of 40–52 doses thrice weekly of poly I:C at 3 $\mu\text{g/g}$ body wt caused very marked potentiation of these parameters for measuring autoimmune response. This effect was minimal when the administration was reduced to six intraperitoneal doses of 1 $\mu\text{g/g}$ given at weekly intervals.

There was far less effect when poly I:C was given intranasally. Forty doses of poly I:C in 0.2 $\mu\text{g/g}$ amount given thrice weekly or six doses of the drug given in 1 $\mu\text{g/g}$ amount at weekly intervals showed no apparent potentiation of glomerular response. There were indications for increased antibody against DNA and sometimes against poly I:C, although in one instance (group 2a) male placebo control animals developed more DNA antibody than did the treated group.

ICR/Ha mice do not develop spontaneous autoimmune disease. The administration of poly I:C to these animals, with the exception of infrequent development of antibody against poly I:C and DNA, appeared to cause no effect with regard to the parameters measured.

Experiments to give precise definition to the degree of potentiation of autoimmune disease in NZB/NZW mice is frustrated by the extreme variability in occurrence of the spontaneous event in terms of different groups of mice and time of appearance. It was necessary, therefore, to assure total randomization of animals for the test and control groups and to use the several parameters measured to judge the effect of the drug.

It is difficult, if not impossible, to extrapolate these data to human populations for purpose of assessing the safety of poly I:C in clinical use. The great majority of persons in the population might be judged to resemble ICR/Ha mice and would likely be unaffected by the drug. The minority of persons who are prone to development of spontaneous autoimmune disorders, such as systemic lupus erythematosus syndrome, might be judged to resemble NZB/NZW mice in some respects. For these individuals, administration of poly I:C parenterally would appear to constitute a real hazard. Administration of the drug in a limited

number of small doses intranasally might be judged to constitute only a minor risk, if any.

One important unanswered question also is whether the induction of interferon is an activity that is separable from the autoimmune stimulation that is readily demonstrable in NZB/NZW mice. Experiments are needed to determine whether other or all inducers of interferon produce this immunologic effect.

Summary. The present report confirms the findings by Steinberg *et al.* (9) that repeated intraperitoneal injections of poly I:C (3 $\mu\text{g/g}$, three times per wk, 40–52 doses) enhanced the incidence and severity of glomerular lesions that occur spontaneously in NZB/NZW mice and also increased the development of circulating antibody against nucleic acids. This effect was minimal when only six intraperitoneal doses were given in 1 $\mu\text{g/g}$ amount at weekly intervals. Intranasal administration of poly I:C (0.2 $\mu\text{g/g}$, three times per wk, 40 doses) or six doses of the drug (1 $\mu\text{g/g}$ weekly) caused no apparent potentiation of glomerular response. ICR/Ha mice, which do not suffer from the spontaneously occurring disease, were unaffected by poly I:C treatment except for occasional development of antibody against poly I:C or DNA.

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