

Distribution of Antibodies to Native DNA, Single-Stranded DNA, and Double-Stranded RNA in Mouse Serums¹ (35347)

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NZB and NZB/W F₁ mice spontaneously develop a disease resembling human systemic lupus erythematosus (SLE). In the human disease antibodies to native DNA are a characteristic finding (1-3), and they appear to be involved in the initiation of renal injury (4, 5). Previous studies have indicated that similar antibodies are found in NZB/W mice (6-8); however, less information is available concerning the specificity of these antibodies in various strains of mice. Recently it was demonstrated that antibodies reactive with double-stranded DNA are closely restricted to SLE patients while antibodies to single-stranded DNA and RNA are found in other diseases as well (9). The possibility that antibodies to these polynucleotides might be present in multiple strains of mice was suggested by their occurrence in several human diseases characterized by infection or active tissue destruction. The present study indicates that, while antibodies to native DNA occur selectively in NZB/W mice, antibodies to other polynucleotides are more widely distributed among different strains of mice. The antigenic potential of synthetic double-stranded RNA in mice is also demonstrated.

Materials and Methods. NZB, NZB/W F₁ hybrid, and SWR mice were obtained from colonies maintained at the Rockefeller University. Strain RF/Un mice were obtained from Simonsen Laboratories, Gilroy, California. All other mice were obtained from Jackson Laboratories, Bar Harbor, Maine.

Hemagglutination procedures as previously

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described (9) were used for the detection of antibodies to native DNA, single-stranded DNA, and double-stranded RNA. The specificity of the reaction was tested by blocking experiments. Antibodies to native DNA and poly A·poly U were blocked by native DNA and poly A·poly U, respectively. For detection of single-stranded DNA antibodies, serums were absorbed with excess native DNA. Additional specificity was shown by rabbit antisera prepared to complexes of single-stranded DNA and methylated bovine serum albumin. These rabbit antisera reacted only with cells coated with single-stranded DNA, but not with native DNA. Special precautions were taken with mouse serum to prevent release of DNA from cell breakdown. Cell hemolysis was avoided and all cells were removed immediately from the serum specimens. Without these steps release of DNA was often observed.

Five-month-old SWR female mice were immunized intraperitoneally with poly A·poly U or poly I·poly C. Mice were divided into two groups and each mouse received 100 µg of one of the polynucleotides in phosphate buffered saline. The immunization schedule consisted of eight weekly injections. After a rest period nine additional injections were given every other day.

In a second experiment, SWR mice were given intraperitoneal injections of poly I·poly C with native DNA, poly I·poly C with single-stranded DNA, single-stranded DNA, or native DNA in phosphate buffered saline. Each mouse received 100 µg of each antigen used. A total of 10 injections were given on an alternating day schedule. Animals were bled periodically by orbital sinus puncture.

Results. Distribution. The distribution of hemagglutinating antibodies to DNA in

TABLE I. Distribution of Antibodies to DNA in NZB/W F₁ Mice.

Age (months)	Native DNA				Single-stranded DNA			
	Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%
15	6/15	40 (5.7) ^a	2/6	33 (4.0)	7/15	47 (6.0)	3/6	50 (5.0) ^a
12	1/18	6 (4.0)	4/19	21 (6.0)	2/18	11 (4.0)	5/9	26 (5.0)
8	0/28	0	6/30	20 (5.3)	1/17	6 (4.0)	11/30	37 (4.7)
5	0/10	0	1/10	10 (5.0)	1/10	10 (4.0)	5/10	50 (4.8)
2	0/5	0	0/5	0	1/9	11 (4.0)	3/9	33 (5.0)

^a Mean titer of group of serums expressed as log base 2, shown in parentheses.

NZB/W F₁ mice of various ages is shown in Table I. Antibodies to native DNA appear earlier in female mice and the incidence of these antibodies increases with age. Over one-third of the 15-month-old mice have antibodies to native DNA. In this older group the incidence of native DNA antibodies was similar in both sexes, but they were rarely seen in younger male mice. Antibodies to single-stranded DNA appeared earlier and were found more frequently than native DNA antibodies. Half of the 15-month-old mice had antibodies to this polynucleotide.

The frequency of antibodies to DNA in eight strains of 7–12-month-old female mice are shown in Table II. Antibodies to native DNA were found in one-fifth of 12-month-old female NZB/W mice as indicated above. These antibodies were not found in other strains except for the rare occurrence in older A/J and A/HeJ mice.

Antibodies to single-stranded DNA were

found in five of the eight strains studied and they did not appear more frequently in NZB/W and NZB mice than in three other strains of mice tested. Table II also shows the results of analysis for antibodies to double-stranded RNA. Thirty percent of 1-year-old female NZB and A/HeJ strains had antibodies to poly A•poly U. These antibodies were also found to a lesser extent in other strains of mice. These serums were also tested with poly I•poly C coated cells and there was generally less reactivity than with poly A•poly U. Serums from C57BR/6J, SWR, and RF strains rarely contained antibodies to these polynucleotides.

Immunization. The results of immunization of mice with poly A•poly U and poly I•poly C without adjuvant or protein carrier are shown in Fig. 1. Five of the 12 mice developed hemagglutinating antibodies reactive with poly I•poly C and 4 of 12 had antibodies reactive with poly A•poly U after

TABLE II. Distribution of Antibodies to Polynucleotides in Several Strains of Female Mice.

Strain	Age (months)	Positive (%)					
		Native DNA		Single-stranded DNA		Poly A • poly U	
		No.	%	No.	%	No.	%
NZB/W	12	4/19	21 (6.0) ^a	5/19	26 (5.0)	2/10	20 (4.0)
NZB	12	0/20	0	5/15	33 (4.0)	6/20	30 (4.0)
A/J	8–12	1/25	4 (4.0)	6/22	27 (5.5)	2/10	20 (5.0)
A/HeJ	7–12	1/23	4 (4.0)	5/23	22 (6.0)	3/10	30 (5.5)
C57BL/6J	12	0/13	0	8/18	44 (5.8)	1/10	10 (4.0)
C57BR/6J	12	0/8	0	0/8	0	0/8	0
SWR	12	0/10	0	0/15	0	0/13	0
RF/U _n	12	0/12	0	0/12	0	1/12	8 (4.0)

^a Mean titer of group of serums expressed as log base 2.

multiple biologic actions including interferon production, cytotoxicity, adjuvant effect, and immunogenic potential. The data reported here show that both poly A·poly U and poly I·poly C are potent immunogens in mice without the use of Freund's adjuvant or *in vitro* binding to a protein carrier. These sera did not contain antibodies to DNA as has been shown to occur in NZB/W F₁ female mice after immunization with poly I·poly C (13). It is also known that RNA and DNA containing viruses can accelerate the production of DNA antibodies and nephritis in NZB/W mice (14). Both single-stranded DNA when coupled to a protein carrier and double-stranded RNA are immunogenic in animals, while the immunogenicity of native DNA has not been clearly demonstrated. The factors eliciting native DNA antibodies in the NZB/W F₁ mice are unknown, but RNA and DNA viruses as well as synthetic double-stranded RNA appear capable of augmenting the response and stimulating disease activity. It seems likely that a specific genetic predisposition for formation of antibodies to native DNA may occur in NZB/W F₁ mice and in human SLE.

Summary. Hemagglutinating antibodies to native DNA were found to occur selectively in NZB/W mice and rarely in other strains. In contrast, antibodies to single-stranded DNA and double-stranded RNA have a more widespread distribution. They occur in NZB and NZB/W mice as well as other strains of

mice. Poly A·poly U and poly I·poly C are shown to be immunogenic in mice without the use of Freund's adjuvant or *in vitro* coupling to a protein carrier.

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