Antibodies to Polynucleotides: Distribution in Human Serums

Abstract. *Hemagglutination procedures were used to determine the distribution of antibodies to native DNA, single-stranded DNA, and double-stranded RNA. Antibodies to all three polynucleotides were found in a high percentage of the serums of patients with systemic lupus erythematosus. Antibodies to native DNA occurred almost exclusively in serums of patients in the active stages of systemic lupus erythematosus, whereas antibodies to single-stranded DNA were observed in the serums of patients with several diseases and of some normal individuals.*

Antibodies reactive with single-stranded DNA may be induced in experimental animals (*1, 2*), whereas the antigenicity of native DNA has not been demonstrated. Immunological studies in man indicate that antibodies to native DNA have considerable specificity for systemic lupus erythematosus (SLE) (*3*) and play a significant role in the renal lesions (*4*). There is a relative paucity of information concerning the distribution of antibodies to single-stranded DNA, and in most instances where such measurements have been made (*5*) the specificity of the reaction for single-stranded DNA was not entirely clear. Considerable interest is focused on the mechanism of production of antibodies to native DNA in patients with SLE. It seemed possible that some type of altered DNA may be presented to the immune system and induce antibodies to both native and single-stranded DNA (*6*). The occurrence of antibodies to polynucleotides in SLE compared to other diseases and the temporal relation between the titers of these antibodies in SLE serums have been studied in order to evaluate the importance of antibodies to native DNA.

Our modification of the hemagglutination test permits the detection of antibodies specific for single-stranded DNA (*7*), double-stranded RNA (poly A · poly U) and native DNA (*8*) so that a large number of serums from patients with SLE and other diseases can be assayed. Formalinized, tanned, human red blood cells (type O, Rh positive) were coated with single-stranded DNA labeled with tritiated actinomycin D by incubation with McIlvaine's buffer (*p*H 4.7) at 37°C (*9*). Actinomycin labeling decreased the nonspecific agglutination which red blood cells coated with single-stranded DNA exhibited and was useful for detecting the amount of antigen bound to cells. Incubation periods longer than 15 minutes did not significantly increase the amount of single-stranded DNA coating the cells and decreased the reactivity of the coated cells. Test serums were inactivated at 56°C for 30 minutes and absorbed with excess native DNA. The absorption procedure allowed the detection of antibodies which reacted only with cells coated with single-stranded DNA. Cells coated with native DNA and double-stranded RNA were prepared by incubating the antigen with formalinized, tanned, red blood cells for 60 minutes in McIlvaine's buffer at *p*H 4.9. Reactions of antibody to native DNA were blocked by either single-stranded or native DNA, whereas antibody to single-stranded DNA was inhibited only by single-stranded DNA. Additional evidence for the specificity of the hemagglutination reaction for single-stranded DNA was obtained by the positive reaction of rabbit antiserums prepared to conjugates of single-stranded DNA and methylated bovine serum albumin (*1*), of adenosine and bovine serum albumin, and of thymidine and bovine serum albumin (*2*), which showed no reactivity with cells coated with native DNA. Antibody to double-stranded RNA was inhibited by poly A · poly U.

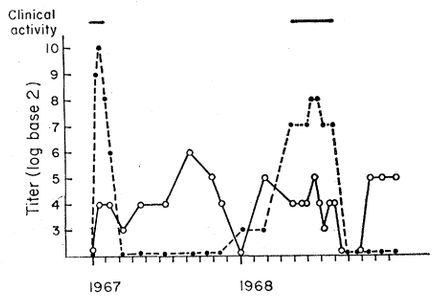

Fig. 1. Independent variation of the titer of antibody to single-stranded DNA and antibody to native DNA during the course of SLE in a patient. Peaks of antibody to native DNA occurred only during the periods of clinical activity, whereas antibody to single-stranded DNA antibody showed a more sporadic variation. Solid circles, native DNA; open circles, single-stranded DNA.

Hemagglutinating antibodies to native DNA were found in 60 percent of SLE serums (Table 1). All patients with antibodies to native DNA had active SLE. In contrast, normal control serums, random serums obtained from hospital patients, and serums from patients with other diseases rarely contained antibodies reactive with cells coated with native DNA. The titer of antibodies in other than SLE subjects was low, and hemagglutination patterns were weak in comparison with those of SLE serums. Antibodies to native DNA were not prominent in the serums of patients with the diseases shown in Table 1, despite the high incidence of antibodies to nuclear or cytoplasmic antigens prevalent in serums from other than the SLE patients.

Antibodies to single-stranded DNA were observed in 92 percent of SLE serums (Table 1). These antibodies were also found in 3.7 percent of normal serums and 17 percent of serums from random hospital patients. In patients with chronic active hepatitis, rheumatoid arthritis, or infectious mononucleosis the incidence of antibodies to single-stranded DNA was high. These diseases are characterized by infection or active tissue destruction. In a group of patients, procainamide administration, which frequently induces antibodies to nuclear antigens and a syndrome with similarities to SLE, increased the incidence of antibodies to single-stranded DNA. In patients with chronic glomerulonephritis and primary biliary cirrhosis the incidence of antibodies to single-stranded DNA was lower. The average hemagglutination

Table 1. Incidence of antibodies to native and single-stranded DNA.

Serums		Antibodies positive (%)	
Source	No. tested	Native DNA	Single-stranded DNA
SLE	50	60.0 (7.1)*	92.0(5.1)
Normal	280	0.3 (5.0)	3.7(4.0)†
Hospital‡	65	0	16.8(4.3)
Procainamide	14	0	57.1(4.5)
Chronic active hepatitis	43	2.3 (3.0)	58.2(5.1)
Infectious mononucleosis	20	0	40.0(5.7)
Rheumatoid arthritis	32	3.1 (3.0)	59.5(5.5)
Chronic glomerulonephritis	40	2.5 (4.0)	7.5(4.6)
Primary biliary cirrhosis	20	0	15.0(3.6)

* Mean titer of group of serums expressed as log base 2, shown in parentheses. † Of 280 serums, 170 were tested for antibodies to single-stranded DNA. ‡ Random hospital serums obtained from patients with a variety of diseases.

titer was also lower in a group of patients with the last-mentioned diseases. Antibodies to double-stranded RNA were found in 54 percent of 61 SLE serums tested. These antibodies were also found in a low incidence in patients with rheumatoid arthritis, infectious mononucleosis, chronic active hepatitis, and in patients chosen at random as controls.

Radioactive labeled DNA was precipitated with ammonium sulfate (*10*) in order to determine whether antibodies to single-stranded DNA were also detectable by this method. This test revealed an even higher incidence of antibodies to single-stranded DNA than did the hemagglutination assay. However, further studies are required to define this system, since antibodies which react with single-stranded DNA are not as easily inhibited in the ammonium sulfate system as in the hemagglutination assay.

Comparison of the titers of antibody to single-stranded DNA was made in nine patients with SLE during the stages of active disease and during periods of no evident clinical activity. Although the titers of antibody to native DNA and serum complement were closely correlated with clinical exacerbations, antibody to single-stranded DNA persisted during quiescent periods in eight of nine serial studies over periods of 1 to 2 years. Figure 1 illustrates the course of one patient whose serum showed three peaks of antibody to single-stranded DNA. Two peaks occur during periods when antibody to native DNA is not detectable and serum complement is normal. Although the participation of antibody to single-stranded DNA in an antigen-antibody complex system cannot be excluded, the ubiquitous distribution of these antibodies would suggest either that they are not components of an immune complex system of significant cytotoxicity or, alternatively, that circulating single-stranded DNA appears infrequently. Certain periods are noted when a rise in titer of antibody to single-stranded DNA corresponds with an increase in the titer of antibody to native DNA. Our studies are directed toward identifying circulating single-stranded DNA before or after these peaks of antibody activity.

The data presented indicate that a variety of antibodies to polynucleotides (native DNA, single-stranded DNA, and double-stranded RNA) are found in patients with SLE. Although the factors eliciting the formation of these antibodies are unknown, viral nucleotides or breakdown products of DNA may serve as antigens in a variety of diseases as well as SLE. It is possible that a unique determinant of altered DNA occurs in subjects with SLE or that a specific genetic predisposition exists for the formation of antibodies to native DNA.

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8. The term native DNA is used to compare the untreated DNA preparation with that obtained after heating at 100°C for 10 minutes followed by rapid cooling. This is not meant to imply that the native DNA is necessarily in a completely native conformation.
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Buoyancy Control in the Freshwater Turtle, Pseudemys scripta elegans

Abstract. *Freshwater turtles* (Pseudemys scripta elegans) *significantly corrected experimental displacements of their specific gravity. By reciprocally changing the volumes of lung air and stored water, they set their buoyancy and maintained their body volume. The cloacal bursae may be the active site for water storage and exchange in this mechanism.*

The ability of turtles to control their specific gravity has been considered for many years (*1*) but neither a clear experimental demonstration of control nor an adequate description of the mechanism has been given. Control is most apparent to the observer in the semiaquatic freshwater turtles which, despite their heavy shells, maneuver easily through the water, choosing at one moment to float at the surface and at another to rest on the bottom. To confirm experimentally the existence of control in such a turtle, I displaced the variable suspected of control (specific gravity initially, (ii) attach disturbance (weights or floats) and then noted whether the animal could restore the displaced variable to normal.

Female turtles of the species *Pseudemys scripta elegans*, weighing from 400 to 600 g, were studied. The experimental procedure to test for buoyancy control was to (i) determine specific gravity initially, (ii) attach weight or float, (iii) place the turtle in deep water (25 cm or more) for 18 hours (*2*), and (iv) determine final specific gravity. To measure specific gravity, I weighed each turtle in air (to 1 g) on a triple-beam balance and in water (to 0.1 g) by placing the turtle in a wire cage, submerged in distilled water, suspended from the below-table hook of a top-loading balance. Specific gravity was experimentally increased by taping a lead weight to the plastron (approximately doubling the turtle's weight in water) and was decreased by taping a Styrofoam float to the carapace (about counterbalancing the weight in water). Two groups of eight turtles each were tested with both weights and floats. Turtles in the first group were intact; those in the second, or cloaca-occluded group, each had a soft rubber plug tied securely into its cloaca.

The intact turtles significantly adjusted their specific gravity toward the initial value in response to both weights and floats; the cloaca-occluded turtles successfully compensated only for the floats (Table 1). These results confirm the existence of buoyancy control in this species and also suggest the mechanism. In each adjustment the body weights in water and in air