observed with the single cell assays (Table 1).

Inactivation of enzyme activity by heat or surface denaturization abolished its effect (Table 1). Furthermore, simultaneous administration of 10 mg of asparagine daily to each animal, along with the enzyme, generally reversed the inhibitory effect of the enzyme.

Treatment of mice with L-asparaginase at the time of immunization may interfere with formation of antibody by individual cells. It seems unlikely that the enzyme affects the immune response merely by destruction of leukocytes or their precursors. A significant decrease in spleen size and cell number occurred only after multiple injections of the enzyme. Cessation resulted in a rapid restoration of the spleen weight.

The effect of enzyme treatment on antibody-forming cells could be due to "starvation" of rapidly dividing cells involved in the immune response, a mechanism similar to that proposed for the suppression of leukemic cells by asparaginase (*2*). Maintenance of relatively high concentrations of this enzyme in the blood reduces the amount of circulating asparagine (*1*, *2*). Lymphocytes and plasma cells involved in the immune response would thus be unable to obtain a sufficient quantity of this specific amino acid and would be unable to participate in the complex sequence of cellular and chemical events involved in antibody synthesis. Reversal of the inhibitory effect of asparaginase by large doses of asparagine is compatible with this hypothesis. The possibility also exists that the immunosuppressive effect of this enzyme from *E. coli* is due to nonspecific "antigenic competition" or to toxic properties of bacterial endotoxins, which undoubtedly contaminate the preparation. However, it would be expected that the two last-mentioned mechanisms would result in immunosuppression only when enzyme was injected before and not after injection of red blood cells (*5*).

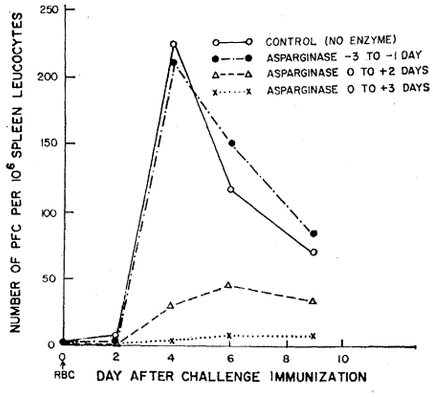

Fig. 1. The cytokinetics of the antibody plaque response to sheep erythrocytes in spleens of control mice and mice treated with *E. coli* L-asparaginase (10 I.U.) either before or after injection of red blood cells. Each point represents the average number of PFC's per 10^6 spleen cells of five or more mice. All animals were immunized intraperitoneally with 4×10^8 red blood cells (RBC) on day 0.

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Tolerance to Polyinosinic • Polycytidylic Acid in NZB/NZW Mice

Abstract. *Immunological tolerance to polyinosinic • polycytidylic acid was induced in NZB/NZW mice. This is the first experimental induction of tolerance to a nucleic acid antigen.*

The disease of NZB/NZW F_1 (B/W) mice is a useful model for human systemic lupus erythematosus. Both mice and humans develop antibodies to DNA, deposit DNA-containing immune complexes in their kidneys, and die of glomerulonephritis. The murine disorder is strongly influenced by genetic factors (*1*). These factors may operate through altered immunologic regulation leading to augmented antibody responses and inability to develop or maintain immunologic tolerance (*2*). These mice carry murine leukemia virus and make antibodies to viral antigen (*3*).

Treatment of B/W mice with multiple injections of fluid polyinosinic • polycytidylic acid (poly I • poly C) caused production of interferon, but the mice became immunized to the double-stranded RNA and had an accelerated onset of antibodies to DNA and of nephritis (*4*). Control mice treated with fluid poly I • poly C did not make antibodies to RNA or DNA.

Because antibodies to nucleic acids are important in the pathogenesis of murine and human systemic lupus erythematosus (*5*), we considered the induction of specific immunological tolerance or suppression to prevent their formation. Both corticosteroids and cyclophosphamide are efficacious in the therapy of New Zealand mice (*6*). The therapeutic approach of specific tolerance in systemic lupus erythematosus might avoid many of the problems of toxicity and infection that occur with standard current therapy, long-term corticosteroids, or immunosuppressive agents. The finding that poly I • poly C acts as a specific in-

Table 1. Tolerance to poly I • poly C in 4-week-old female B/W mice given cyclophosphamide (60 μg/g) 24 hours after receiving fluid poly I • poly C (100 μg) and challenged with poly I • poly C in complete Freund's adjuvant. The untreated controls were not challenged with poly I • poly C in complete Freund's adjuvant.

Treatment	Mice (No.)	Poly I • poly C binding capacity (μg/ml)
Poly I • poly C + cyclophosphamide		
3 courses	8	< 0.1
2 courses	8	< 0.1
1 course	8	1.1
Cyclophosphamide alone	12	11.0
Poly I • poly C	12	12.4
Controls	6	11.5
Untreated controls	16	0.1

ducer of antibodies to nucleic acids made it possible to pursue these therapeutic concepts.

The administration of cyclophosphamide 24 hours after antigen administration leads to a state of specific immunologic tolerance, apparently by destroying rapidly proliferating sensitized cells (*7*). Animals treated in this manner show a diminished response to subsequent antigenic challenge. We treated a group of B/W female mice (1 month old) with a single injection of poly I • poly C (100 μg) followed on the next day by 60 mg of cyclophosphamide per kilogram of body weight intraperitoneally (one course). In other groups of B/W females (1 month old) this was repeated two or three times separated by 4 to 5 days. Control mice were treated with either cyclophosphamide alone (60 mg/kg) two or three times, or with poly I • poly C alone (100 μg) two or three times. All mice were challenged with poly I • poly C in complete Freund's adjuvant 11 days after the last injection of cyclophosphamide. Equal volumes of poly I • poly C (1 mg/ml) and complete Freund's adjuvant were emulsified, and 0.3 ml of the mixture injected intraperitoneally. The mice were bled 2 weeks later for assay of antibodies to RNA.

Serum poly I • poly C binding was determined by a modification of the Farr technique with ^{14}C-labeled poly I • poly C (Miles Laboratories) as antigen and with 35 percent ammonium sulfate in the final reaction mixture (*4*). Each serum was titered with the ^{14}C-labeled poly I • poly C. The 50 percent binding point was considered the antigen-binding capacity of the serum. Animals that received two or three courses of poly I • poly C and cyclophosphamide made no detectable antibody to poly I • poly C after challenge (Table 1). By contrast, animals treated with either poly I • poly C alone or with cyclophosphamide alone were immunized by the challenge dose. Thus, the combined use of poly I• poly C and cyclophosphamide in this schedule led to a profound state of immunologic tolerance. A single course led to a 90 percent reduction in binding capacity. The specificity of the tolerance to poly I • poly C was demonstrated by the normal response to immunization with sheep erythrocytes of animals made tolerant to poly I • poly C.

This is the first demonstration of experimentally induced tolerance to a nucleic acid antigen. The effectiveness of this therapy in either the prophylaxis or therapy of murine or human systemic lupus needs to be determined. This technique may have widespread application in various "autoimmune" diseases as well as in the field of transplantation.

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Cystic Fibrosis: Characterization of the Inhibitor to Ciliary Action in Oyster Gills

Abstract. *The inhibitor to oyster ciliary activity was isolated from serum of cystic fibrosis patients and heterozygotes. The inhibiting protein fraction was a cation as judged by electrophoresis; it had a molecular weight of 125,000 to 200,000 as judged by gel filtration; and on diethylaminoethyl-cellulose chromatography it eluted with the immunoglobulin G fraction. The analogous fraction in normal individuals did not inhibit oyster ciliary activity.*

Serums from cystic fibrosis (CF) patients and their parents inhibit ciliary activity in preparations of rabbit trachea (*1*), oyster gills (*2*), and freshwater mussels (*3*).

We report the characterization of the serum fraction that inhibits oyster gill cilia. The results from separations on preparative starch-block electrophoresis, on diethylaminoethyl (DEAE) cellulose chromatography, and on Sephadex G200 gel filtration indicated that the inhibiting factor is present in the γ-globulin fraction of serum from CF

Table 1. Time required by electrophoretic serum fractions to stop cilia. Fractions designated "N.T." were not tested because they were not properly separated by electrophoresis or because they developed precipitates. "Ex" indicates fractions that produced expulsion of debris.

Subjects	Time required (min)					
	Whole serum	Fractions				
		G	E	D	C	B
			Patients			
E. D.	10	60	55	50	44	25 Ex
J. A.	8	N.T.	N.T.	60	40	15 Ex
J. B.	15	N.T.	50	15	55	20 Ex
P. C.	10	55	N.T.	50	45	5 Ex
M. E.	12	50	35	0	55	22 Ex
J. W.	15	50	40	50	40	30 Ex
J. L. M.	10	45	50	N.T.	40	2 Ex
S .S.	20	50	50	50	50	1 Ex
S. T.	20	33	45	45	40	30 Ex
P.P.	N.T.	65	N.T.	50	50	22 Ex
			Heterozygotes			
Mrs. W.	20	45	50	50	50	24 Ex
Mr. W.	15	25	55	50	40	35 Ex
Mrs. D.	17	N.T.	33	N.T.	60	38 Ex
Mrs. M.	8	40	40	50	N.T.	18 Ex
			Presumed heterozygote			
M. L. M.	15	50	50	50	30	7 Ex
			Normals			
L. L.	50	50	42	50	50	50
P. B.	50	25	16	N.T.	50	50
D. B.	50	50	50	50	50	50
L. N.	50	35	50	47	50	47
B. J.	48	60	60	60	60	41
M. K.	50	N.T.	N.T.	N.T.	40	50