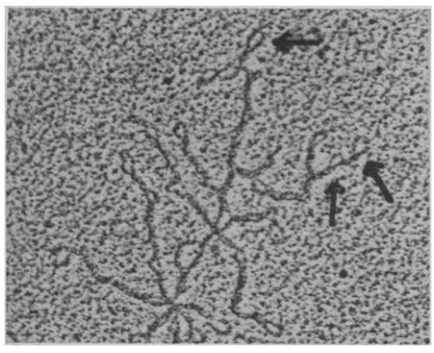

Fig. 3. Highly supercoiled chloroplast DNA. Arrows point to loops (about × 75,000).

that the DNA is circular, as it could be the result of replicative unwinding (*8*). Although few or no free ends could be seen, it was impossible to say that the tangled mass of a display consisted of only one molecule.

Acetabularia chloroplasts did not release anything resembling the single-stranded "mesh" DNA of spinach chloroplasts (*9*). Although strands about 15 Å in diameter were seen in some displays, they were always near other strands or stroma debris, whose effect on width of rotary-shadowed DNA is unknown. We did not see anything resembling the "hybrid loops" reported for *A. crenulata* chloroplast DNA (*10*).

Between 20 and 40 percent of the ruptured chloroplasts had DNA associated with them. The percentage depended on the degree of intactness of the chloroplast preparation. This is probably due to the presence of an intrachloroplastal nuclease, because chloroplasts incubated in buffered sucrose at 37°C for half an hour before spreading released only short fragments. The addition of deoxyribonuclease just before spreading produced the same effect. In addition, some of the DNA could be lost into the hypophase.

Attempts to isolate intact molecules by using phenol extraction resulted in extensive breakage. The largest amount of DNA released by osmotic shock which was untwisted enough to measure was 419 μm in length, corresponding to a molecular weight of 805×10^6 daltons (*11*). This suggests that the amount of DNA per chloroplast is in the same range as that of bacteria (*2, 3*) and within the range of DNA content determined chemically for chloroplasts of *Chlorella* (*12*), *Chlamydomonas* (*13*), *Euglena* (*14*), and lettuce (*15*). This amount is much greater than that of a mitochondrion (*1*). Whether the chloroplast DNA consists of unique sequences or highly redundant ones (that is, its information content) will have to be determined by renaturation kinetics (*15*).

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References and Notes

1. M. M. K. Nass, *Science* **165**, 25 (1969).
2. R. Bode and H. Morowitz, *J. Mol. Biol.* **23**, 191 (1967).
3. L. A. MacHattie, K. I. Berns, C. A. Thomas *ibid.* **11**, 648 (1965).
4. D. C. Shephard, *Exp. Cell Res.* **37**, 93 (1965).
5. B. R. Green, V. Heilporn, S. Limbosch, M. Boloukhere, J. Brachet, *Proc. Nat. Acad. Sci. U.S.* **58**, 1351 (1967).
6. L. Lateur, *Rev. Algol.* **1**, 26 (1963).
7. A. K. Kleinschmidt, *Meth. Enzymol.* **12B**, 361 (1968).
8. C. O. Person and D. T. Suzuki, *Can. J. Gen. Cytol.* **10**, 627 (1968).
9. C. F. L. Woodcock and H. Fernandez-Moran, *J. Mol. Biol.* **31**, 627 (1968).
10. G. Werz. and G. Kellner, *J. Ultrastruct Res.* **24**, 109 (1968).
11. M. H. F. Wilkins, *Science* **140**, 941 (1963).
12. T. Iwamura and S. Kuwashima, *Biochim. Biophys. Acta* **174**, 330 (1969).
13. K.-S. Chiang and N. Sueoka, *Proc. Nat. Acad. Sci. U.S.* **57**, 1506 (1967).
14. G. Brawerman and J. M. Eisenstadt, *Biochim. Biophys. Acta* **91**, 477 (1964).
15. R. Wells and M. Birnsteil, *Biochem. J.* **112**, 477 (1969).
16. Supported by National Research Council of Canada grant A4688. We thank Dr. T. Bisalputra for helpful advice and the use of the Zeiss EM9A, L. Veto for assistance with the Hitachi HU-11A, and D. Bray for photographic printing.

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Myxovirus Antibody Increases in Human Connective Tissue Disease

Abstract. *Antibodies to measles and parainfluenza type 1 viruses were significantly increased in systemic lupus erythematosus and Reiter's syndrome. Of the individuals with highest titers of measles antibody, 75 percent had neurologic illness. Persistent virus infection may be a factor in the pathogenesis of these diseases.*

The human connective tissue diseases are characterized by abnormal immunologic processes, which appear, in part, to mediate pathogenesis. However, the stimulus initiating this abnormal response, and hence the etiology of these diseases, remains obscure. There has been renewed interest in the role of microbial agents as initial, and perhaps persisting, etiologic factors (*1*). A possible parallel exists in the evolution of knowledge about subacute sclerosing panencephalitis (SSPE), where initially myxovirus-like inclusions were found in brain tissue by electron microscopy, next the titers of serum antibodies to measles were shown to be increased, and then measles virus was isolated from brain (*2*). In view of these findings, the discovery of similar microtubular inclusions in renal tissue from a patient with systemic lupus erythematosus (SLE) was of interest (*3*). These morphologic observations have been confirmed and extended, but their real nature and specificity is unclear (*4*). Measles and the other viruses known to cause such inclusions belong to the paramyxovirus group. We now report on the concentration of antibodies to two of these viruses in patients with connective tissue diseases.

Patients were divided into groups according to their clinical diagnoses. The SLE group was further subdivided into those with (SLE + N) and those without (SLE − N) neurologic symptoms or signs, since an earlier study suggested that the titer of antibodies to measles was higher in the former (*5*). Of the six patients with Reiter's syndrome, four had all three typical characteristics (arthritis, urethritis, and conjunctivitis). The rheumatoid arthritis group contained 15 patients with classical or definite, and five with probable, rheumatoid arthritis. The juvenile rheumatoid arthritis group contained three patients with definite, and four with possible, disease. The other connective tissue disease group had six with progressive systemic sclerosis, five with vasculitis, two each with dermatomyositis, "overlap" syndrome (features of several distinct connective tissue diseases), and Dilantin-induced SLE, and one with arthritis and hypogammaglobulinemia. The miscellaneous group included ten patients with arthritides other than rheumatoid arthritis, eight members of two families in each of which several children had polyarthritis of undetermined etiology, four patients with undiagnosed neurologic illness, and ten with various other conditions. Serums were obtained from three patients with SSPE (*6*). The normal group was drawn from physicians and laboratory personnel. Serums were stored at −20°C. Subsequent analysis

of the sex, race, and age of the subjects in each group showed that females predominated in the SLE, rheumatoid arthritis, juvenile rheumatoid arthritis, other connective tissue disease, and the miscellaneous groups. There were more Negroid than white subjects in the SLE and rheumatoid arthritis groups (71 and 76 percent), equal numbers in the Reiter's syndrome and miscellaneous groups, and fewer in the other groups. Mean age was similar in all groups, except in the juvenile rheumatoid arthritis and rheumatoid arthritis groups which were younger and older, respectively.

Measles antibody was measured by the hemagglutination inhibition method (*7*) modified for microtechnique (*8*). All serums were initially absorbed with 25 percent kaolin and then with rhesus monkey erythrocytes. Measles virus hemagglutinating antigen was grown in a human heart cell line; a 1 percent suspension of rhesus red cells was used in the test (*9*). Five known positive and negative serums and a reference measles antiserum obtained from hyperimmunized monkeys (*10*) were included as standards in each test. The antibody titer was recorded as the reciprocal of the last dilution showing complete or partial hemagglutination inhibition, converted to the $\log_2$ value and adjusted to the titer of the monkey reference serum standard in the same test.

Antibody to parainfluenza type 1 was also measured by the hemagglutination inhibition micromethod (*7, 8, 11*), with the use of commercial reagents (*9*). Serums were absorbed with receptor-destroying enzyme; parainfluenza type 1 hemagglutinating antigen, grown in rhesus monkey kidney tissue culture, and an 0.5 percent suspension of type O human erythrocytes were used. Three known positive and negative serums, a commercial parainfluenza type 1 rabbit antiserum (*9*), and two reference guinea pig antiserums against parainfluenza type 1 strains HA-2/C-39 and Sendai (*12*) were included in each test as standards. The geometric mean titers (and range) of the three animal antiserums were 9.8 (9.3 to 11.3), 6.9 (6.3 to 7.8), and 4.6 (4.3 to 5.3), respectively. Since these standards showed no consistent variation in different tests, the titer of antibody to parainfluenza type 1 was expressed without adjustment as $\log_2$ of the reciprocal of the last serum dilution showing complete or partial hemagglutination inhibition.

Table 1. Measles hemagglutination inhibition antibody in human connective tissue disease. Symbols: SLE, systemic lupus erythematosus; SLE − N and SLE + N, SLE without and with neurologic signs or symptoms; RA, rheumatoid arthritis; JRA, juvenile RA; OCD, other connective tissue diseases; Misc., miscellaneous diseases; SSPE, subacute sclerosing panencephalitis; NS, not significant, $P > .05$; NT, not tested, group too small for analysis.

Diagnosis	Subjects (No.)	Titer ($\log_2$)			*P* to:	
		Geometric mean	Range	S.D.	Normal	SLE
SLE	42	6.31	1.9–11.0	2.42	< .005	
SLE − N	16	6.08	1.9–10.0	2.22	< .02	
SLE + N	26	6.45	2.0–11.0	2.56	< .005	
Reiter's	6	6.00	5.0– 8.0	1.27	< .05	NS
RA	23	4.94	0 – 9.0	2.40	NS	< .05
JRA	7	4.04	0 – 9.3	2.85	NS	< .05
OCD	18	4.92	0 – 8.4	2.39	NS	< .05
Misc.	31	4.85	0 – 9.0	1.92	NS	< .05
SSPE	3	4.93	1.9– 6.6	2.63	NT	NT
Normal	20	4.46	2.3– 7.5	1.64		< .005

All serums were tested against control antigens produced from uninfected tissue cultures of the same kind used to produce the viral antigens, and also without any antigen against the red cells used in the test. In addition to the six serum standards included in each test, many patient serums were tested repeatedly to check the reproducibility of titers. The mean value for each was used for statistical analysis. Two to five specimens obtained at intervals from each of 12 individuals were tested to measure changes in titer; the value for the earliest serum sample from each patient was used for analysis. The geometric mean titers and standard deviations were calculated for each patient group and compared for significant differences by Student's *t*-test (*13*).

The results of the measles antibody study are shown in Table 1. The geometric mean titer of the SLE group was significantly higher than that of all other groups except the Reiter's syndrome and SSPE (the latter being too small a group to analyze statistically). Although the titer of SLE + N was higher than that of SLE − N, the difference was not significant. However, of the 12 patients with the highest titers (≥ 9.0), seven were from the SLE + N, two from the SLE − N, and one each from the rheumatoid arthritis, juvenile rheumatoid arthritis, and miscellaneous-undiagnosed neurologic illness groups. Thus, 75 percent of these patients had neurologic signs or symptoms. One of the SLE + N group had detectable antibody in the cerebrospinal fluid, an unusual finding except in SSPE. Ten of these patients with high titers were adults, mean age 40 (range 22 to 68). These titers were well above the highest of the limited SSPE sample tested.

The only other group showing a significantly higher geometric mean titer than that of the normals was the small Reiter's syndrome sample. Significant differences were not found within any group. However, the mean titer of four patients with undiagnosed neurologic illness was moderately higher (5.90)

Table 2. Parainfluenza type 1 hemagglutination inhibition antibody in human connective tissue disease. Symbols as described in Table 1.

Diagnosis	Subjects (No.)	Titer ($\log_2$)			*P* to:	
		Geometric mean	Range	S.D.	Normal	SLE
SLE	42	8.49	6.3–12.3	1.08	< .001	
SLE − N	16	8.74	7.3–12.3	1.20	< .001	
SLE + N	26	8.34	6.3–10.3	0.99	< .001	
Reiter's	6	8.47	7.3– 9.3	0.75	< .02	NS
RA	20	7.65	6.3–11.3	1.46	NS	< .02
JRA	7	7.73	6.3– 9.3	0.98	NS	NS
OCD	18	7.61	4.3–10.3	1.36	NS	< .01
Misc.	32	7.66	4.3–11.3	1.58		< .02
SSPE	3	8.97	8.3–10.3	1.17	NT	NT
Normal	20	7.03	4.3–10.3	1.27		< .001

than that of the other miscellaneous subgroups (4.41 to 5.13). Samples taken over periods of 2 weeks to more than 5 years (mean 16 months) were measured for each of 12 subjects, including six with SLE. No significant change in titer (> 1 dilution) was found.

Table 2 shows the results of the study on antibody to parainfluenza type 1. The titer of the SLE group was significantly higher than that of the normal group, and of the rheumatoid arthritis, other connective tissue disease, and miscellaneous groups. The SLE + N subgroup was slightly lower than the SLE − N. Of the 11 patients with the highest titers (≥10.3), five were in the miscellaneous group and one each in the SLE − N, SLE + N, rheumatoid arthritis, other connective tissue disease, SSPE, and normal groups. There was no common clinical feature among these patients.

The only other group showing a significant increase over that of the normal group was the Reiter's syndrome group, in which there were only six individuals. Analysis within groups did not reveal significant differences; the undiagnosed neurologic illness subgroup had a similar titer to the other miscellaneous subgroups (7.80 as opposed to 7.65 to 8.00). No significant change in titer was found between serial samples from five individuals (three SLE) spanning 7 months to more than 5 years (mean 24 months). There was no correlation between measles and parainfluenza type 1 titers in individual subjects.

There are at least three possible explanations for the increase in antibodies to measles and parainfluenza type 1 in SLE and Reiter's syndrome. First, they could reflect persistent virus infection. This may, in fact, be the mechanism of the usually lifelong immunity seen after most viral infections. Certainly animals, and to a lesser extent man, harbor many clinically inapparent viruses for long periods (*14*). The immunologic hyperreactivity well known in SLE could then result in increased antibody formation to such viral antigens. However, if a latent virus entered a replicative stage, many immunologic consequences may follow, particularly if host antigens were part of the viral membrane (*15*). Aleutian mink disease, lymphocytic choriomeningitis, and New Zealand mouse disease are examples in animals (*16*). In man the best example is SSPE, where antibody titers to measles virus have generally been above normal (*2*). The 12 patients with the highest measles titers in this study were in the usual SSPE range. The frequency of neurologic involvement in these patients (mostly adult) suggests that persistent measles infection of the central nervous system may not be limited to the children and adolescents in whom SSPE has been found.

Second, the increased antibody levels might be a nonspecific result of immunologic hyperreactivity. In Reiter's syndrome, increase in Bedsonia complement-fixing (CF) antibodies has been reported, but antibodies to viruses were not measured (*17*). Our data suggest that a generalized hyperimmune response may exist in this disease. Such a response does exist in the connective tissue diseases, but there have been few reports on titers of antibody to exogenous antigens. Bacterial antibodies were decreased in SLE, the decrease being primarily in 19*S* antibody (*18*). However, viral hemagglutination inhibition antibody is thought to be primarily 7*S* (*19*). Viral CF antibody levels were decreased in women with positive tests for rheumatoid factor, but no information about the occurrence of arthritis in these subjects was presented (*20*). In another study, CF antibody to herpes simplex was decreased in rheumatoid arthritis patients. No significant differences were found in titers of varicella, adenovirus, cytomegalovirus, or psittacosis-LGV CF antibody, or of rubella hemagglutination inhibition antibody (*21*). Where decreases in CF antibody levels were observed, rheumatoid factor may have played a role.

Finally, the increases in antibody might result from antigens shared by host cell and virus. Paramyxoviruses are enclosed by a membrane, part of which is host cell material (*15*). Thus, one of the autoantibodies produced in SLE might cross-react with a host antigen on the viral membrane. However, this is unlikely because the serums did not react with the control antigen produced in the same system as the viral antigen. An autoantibody to RNA would be unlikely to affect the hemagglutination inhibition reaction because the viral RNA is distinct from the hemagglutination antigen.

Current information does not allow a definitive interpretation of the observed increases in antibody to measles and parainfluenza type 1 viruses in SLE. These data, together with other features of the syndrome, are compatible with the hypothesis that persistent virus infection may underlie the pathogenesis of SLE.

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References and Notes

1. C. L. Christian, *Arthritis Rheum.* **7**, 455 (1964); *Atypical Virus Infections—Possible Relevance to Animal Models and Rheumatic Disease*, C. L. Christian, Ed., *ibid.* **13**, suppl. (in press).
2. J. H. Connolly, I. V. Allen, L. J. Hurwitz, J. H. Millar, *Lancet* **1967-I**, 542 (1967); *Conference on Measles Virus and SSPE*, J. L. Sever and W. Zeman, Eds., *Neurology* **18**, part 2 (1968); F. E. Payne, J. V. Baublis, H. H. Itabashi, *N. Engl. J. Med.* **281**, 585 (1969); L. Horta-Barbosa, D. A. Fuccillo, W. T. London, J. T. Jabbour, W. Zeman, J. L. Sever, *Proc. Soc. Exp. Biol. Med.* **132**, 272 (1969).
3. R. Fresco, *Fed. Proc.* **27**, 246 (1968).
4. S-M. Chou, *Arch. Pathol.* **86**, 649 (1968); F. Gyorkey, K-W. Min, J. G. Sinkovics, P. Gyorkey, *N. Engl. J. Med.* **280**, 333 (1969); W. L. Norton, *J. Lab. Clin. Med.* **74**, 369 (1969); K. Kawano, L. Miller, P. Kimmelstiel, *N. Engl. J. Med.* **281**, 1228 (1969); J. L. Duffy, *ibid.*, p. 562; E. R. Hurd, E. Eigenbrodt, M. Ziff, S. W. Strunk, *Arthritis Rheum.* **12**, 541 (1969).
5. P. E. Phillips and C. L. Christian, *Arthritis Rheum.* **12**, 688 (1969).
6. Kindly provided by D. H. Harter.
7. L. Rosen, *Virology* **13**, 139 (1961).
8. J. L. Sever, *J. Immunol.* **88**, 320 (1962).
9. Obtained from Microbiological Associates, Inc., Bethesda, Md.
10. Available from Division of Biologics Standards, NIH [*USPHS Regulations, Biological Products, Publ. No. 437* (1964), p. 51]. Kindly provided by H. M. Meyer, Jr.; the geometric mean hemagglutination inhibition titer was 12.5, with a range of 11.0 to 13.3 in his laboratory, and 10.3 (range 9.8 to 10.8) in the present study.
11. R. M. Chanock and K. M. Johnson, in *Diagnostic Procedures for Viral and Rickettsial Diseases*, E. H. Lennette and N. J. Schmidt, Eds. (American Public Health Association, New York, 1964), p. 482.
12. Provided by Research Reference Reagents Branch, NIAID, Bethesda, Md.
13. A. B. Hill, *Principles of Medical Statistics* (Oxford, New York, 1966), p. 143.
14. G. D. Hsiung, *Bacteriol. Rev.* **32**, 185 (1968); N. G. Rogers, M. Basnight, C. J. Gibbs, Jr., D. C. Gadjusek, *Nature* **216**, 446 (1967); K. M. Lindgren, R. G. Douglas, Jr., R. B. Couch, *N. Engl. J. Med.* **278**, 517 (1968).
15. K. V. Holmes, H-D. Klenk, P. W. Choppin, *Proc. Soc. Exp. Biol. Med.* **131**, 651 (1969); H-D. Klenk and P. W. Choppin, *Virology*, **38**, 255 (1969).
16. D. D. Porter and A. E. Larsen, *Proc. Soc. Exp. Biol. Med.* **126**, 680 (1967); M. B. A. Oldstone and F. J. Dixon, *Science* **158**, 1193 (1967); R. C. Mellors, T. Aoki, R. J. Huebner, *J. Exp. Med.* **129**, 1045 (1968).
17. T. D. Kinsella, W. L. Norton, M. Ziff, *Ann. Rheum. Dis.* **27**, 241 (1968); J. Schachter, D. E. Smith, R. J. Gilbert, E. P. Engleman, K. F. Meyer, *Arthritis Rheum.* **12**, 329 (1969).
18. J. Baum and M. Ziff, *J. Clin. Inv.* **48**, 758 (1969).
19. S. E. Luria and J. E. Darnell, Jr., *General Virology* (Wiley, New York, 1967), p. 139.
20. M. Waller, J. Sever, N. Curry, M. R. Gilkeson, *Ann. Rheum. Dis.* **25**, 327 (1967).
21. J. D. Smiley and H. L. Casey, *Arthritis Rheum.* **12**, 698 (1969).
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