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Molecular Pathogenesis of Systemic Lupus Erythematosus

By

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With 7 Figures

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Summary

The study of patients with systemic lupus erythematosus revealed the close association of the disease with measles—or a related virus. High titres of antibodies to measles virus were found in patients that correlated with the course of the disease. Immunofluorescence tests revealed measles virus or a related antigen in lupus-affected tissues. Inclusion bodies consisting of paramyxovirus-like ribonucleoprotein structures were regularly detected in both affected tissues and leukocytes. Molecular hybridization of measles virus RNA with DNA from the affected tissues showed that DNA transcripts of measles or a closely related virus are integrated in the cellular nuclear DNA. Possible pathogenetic mechanisms of the disease are discussed.

1. Introduction

Although various concepts on the pathogenesis of systemic lupus erythematosus have been suggested, data are accumulating in favor of the idea, that the disease might be connected with measles virus or a related paramyxovirus infection. The following facts are consistent with this conception. Electron microscopic examination of affected tissues revealed cytoplasmic inclusions containing structures which resembled paramyxovirus-like ribonucleoproteins (1—10), and high titres of antibodies to measles virus were found in patients with systemic lupus erythematosus (11—16). Though several authors claimed the isolation of various viruses from lupus erythematosus patients (17—23) most of them have not been identified as agents of the disease. Attempts to isolate paramyxoviruses, including measles virus, from lupus patients were until recently unsuccessful.

Some authors suppose that systemic lupus erythematosus may be compared with slow virus infections (23, 24) or with neoplastic processes (25). The idea of integration of measles virus genome into cellular DNA has been also suggested by the authors of this paper (26, 27).

This paper presents the results of a study on the viral etiology of systemic lupus erythematosus. Evidence is presented that DNA-transcript of measles virus or a related viral RNA is integrated in the DNA of the affected cells. Some implications of these findings are discussed.

2. Materials and Methods

2.1. Selection of Patients

Patients with systemic lupus erythematosus were observed in the clinical department of the Institute for Rheumatism, Academy of Medical Sciences. Eighty patients at the age of 15 to 50 years and more being taken ill from 3 months to over 20 years were selected for this study. The clinical course of the disease in the patients under study has been described elsewhere (27—30). Patients with other diseases and healthy blood donors were also selected for a comparative study.

2.2. Virus-Specific Antibodies

The following virus antigens were used: measles, rubella, influenza A 2 (Singapore and Hong Kong) and B, adenoviruses types 3 and 7, paramyxoviruses types 1, 2 and 3, respiratory-syncytial (RS) virus, coronavirus (the OS-43 strain), *Mycoplasma pneumoniae* and *M. hominis*, and Australia (SH) antigen.

Immunologic reactions with sera from lupus erythematosus patients and control sera were performed with the above mentioned commercial viral antigens using hemagglutination-inhibition (HI) test for influenza, parainfluenza, measles, rubella viruses, complement fixation (CF) test for other viruses and mycoplasma, and agar gel precipitation test for SH antigen.

2.3. Immunofluorescence Method

Direct immunofluorescence method (31) was used for detection of measles antigens in tissues and leukocytes from lupus erythematosus patients.

Isothiocyanate fluorescein labeled (ITCF)-gamma-globulin was prepared from a serum of a lupus erythematosus patient taken at the acute phase of the disease, that had a high level (1:640) of antibodies to measles virus in hemagglutination-inhibition test.

Blood smears of patients were fixed with acetone, stained with the labeled gamma-globulin in a wet camera at 37° C for 30 minutes. The preparations were then examined in a ML 2 luminescent microscope and photographed.

2.4. Electron Microscopy

Pathologic tissues taken by biopsy or post mortem were fixed by the method of CAULFIELD (32), dehydrated with graded alcohols, embedded into araldite and stained with 1 per cent uranyl acetate in 70 per cent alcohol. Ultrathin sections were prepared with a LKB 4801 A ultratome, contrasted with lead citrate by the method of Reynolds (33) and examined in a JEM 7 electron microscope at the instrumental magnification of 2000× to 7000× times.

2.5. Molecular Hybridization

The preparation of ^{3}H-labeled RNA of measles virus, of nuclear DNA from tissues and the method of molecular hybridizytion of ^{3}H-RNA with DNA are described elsewhere (34).

3. Results

3.1. Serologic Study

The results obtained with various viral antigens and sera of patients with lupus erythematosus, or other diseases and healthy donors are presented in Table 1.

Table 1. *Presence of Antibodies to Various Antigens in Sera of Lupus Erythematosus and Control Patients (HI Tests)*

Diagnosis	Number of patients	Measles	Rubella	Influenza A2	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3
Lupus erythematosus	80	97.5[a]	98.1	58.6	86.6	32.0	60.0	37.3
Rheumatism and rheumatoid arthritis	88	96.7	90.8	65.7	79.6	52.8	50.5	50.3
Systemic sclerodermia	26	92.3	53.3	87.5	75.0	43.7	75.0	50.0
Infectious allergic myocarditis	10	80.0	87.5	62.5	75.0	12.5	25.0	37.5
Chronic hepatitis	32	82.8	n.t.[b]	87.4	90.6	90.6	90.6	62.4
Healthy donors	59	86.5	100	100	100	50.8	96.6	67.8

[a] Per cent positive.
[b] Not tested.

Table 2. *Mean Antibody Titres to Viral Antigens in Sera of Lupus Erythematosus and Control Patients (HI Tests)*

Diagnosis	Number of patients	Measles	Rubella	Influenza A2	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3
Lupus erythematosus	80	370[a]	62	74	305	80	54	40
Rheumatism and rheumatoid arthritis	88	216	75	74	150	118	52	80
Systemic sclerodermia	26	228	36	69	183	16	39	18
Infectious allergic myocarditis	10	116	30	27	58	160	17	26
Chronic hepatitis	32	40	n.t.[b]	70	48	28	38	12
Healthy donors	59	79	48	149	146	25	33	42

[a] Denominator of antibody titres.
[b] Not tested.

Table 3. *Mean Antibody Titers to other Antigens in Sera of Lupus Erythematosus and Control Patients (CF Tests)*

Diagnosis	Number of patients	Adeno-virus	RS virus	Corona-virus	Mycoplasma pneumoniae	Mycoplasma hominis
Lupus erythematosus	78	6.4[a]	5.3	15	23	64.9
Rheumatism and rheumatoid arthritis	67	0.9	1.2	1.9	15	11
Systemic sclerodermia	27	3.0	6.2	1.4	19	2.3
Infectious allergic myocarditis	10	1.0	1.0	0	18	2.0
Chronic hepatitis	32	18	5.7	7.2	53	7.3
Healthy donors	56	3.1	1.5	4.0	53	6.4

[a] Denominator of antibody titers.

It is seen from the Table that antibodies to measles virus and other viruses tested were found in all sera which is not surprising since these virus infections are widely spread among the population. Therefore mean serum titres to the tested viral antigens were calculated for each group of the patients (Table 2). It is seen from the Table that the mean antibody titres to various viral antigens vary in different groups of patients, but that the antibody titre to measles virus is highest in lupus erythematosus patients, particularly when compared with the respective antibody titre in healthy donors.

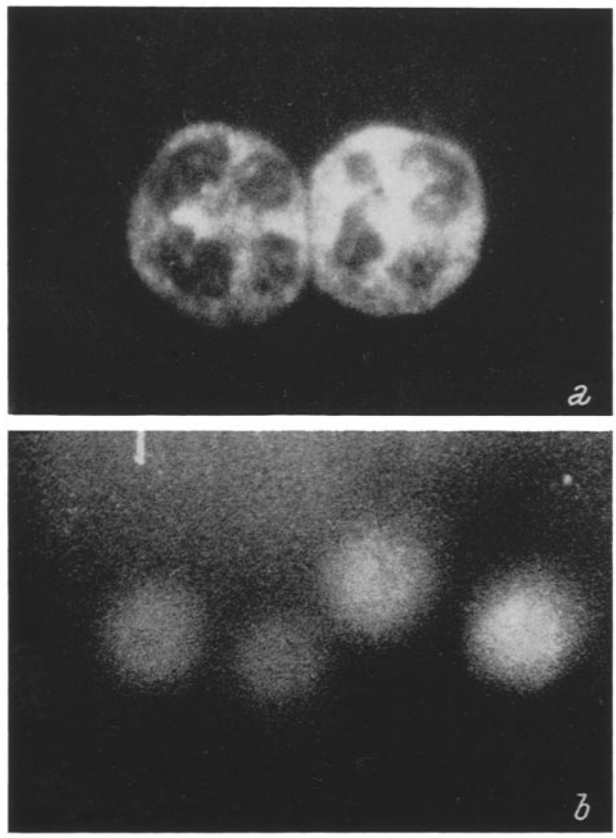

Fig. 1. Immunofluorescence of leukocytes from a patient with lupus erythematosus (a) and from a healthy donor (b)
The blood smears were stained with ITCF-labeled gamma globulin from a lupus erythematosus patient having a measles antibody titre of 1:640

As shown in Table 3, there were rather low antibody titres to other viral and mycoplasma antigens, with the exception of the relatively high antibody titres to *M. pneumoniae* in patients with chronic hepatitis and in healthy donors. Antibodies to SH antigen were detected in two patients, *i.e.* one with lupus erythematosus and one with acute rheumatism.

3.2. Immunofluorescence Tests

Blood smears from 16 patients with lupus erythematosus, 8 patients with rheumatism and rheumatoid arthritis, 2 patients with systemic sclerodermia, 1 patient with dermatomyositis and 7 healthy donors were studied in immunofluorescence tests with ITCF-labeled gamma globulin containing a high titre of measles antibody. Measles antigen was revealed in 14 smears (88 per cent) from lupus erythematosus patients and in 5 smears (27 per cent) from control patients, the latter including 3 positive results in patients with systemic sclerodermia, dermatomyositis and rheumatoid arthritis.

Immunofluorescence of leukocytes from lupus erythematosus patients was intensive and localized in the cytoplasm. The amount of fluorescent cells reached 80 per cent, whereas immunofluorescence of leukocytes from healthy persons was dubious or negative (Fig. 1).

3.3. Electron Microscopic Examination

Inclusions resembling paramyxovirus-like ribonucleoprotein structures were revealed both in the cytoplasm and in the nuclei of lupus erythematosus tissues.

Figure 2 presents an endothelial cell with a cytoplasmic inclusion consisting of tubular structures. These structures are better resolved in Figure 3, their external diameter is about 220 Å. Nuclear inclusions differ from those in cytoplasm in that: they represent structureless bodies (Fig. 4), although sometimes tubular structures are revealed too (Fig. 5).

Tubular structures were found in tissues of 19 patients with lupus erythematosus *i.e.* in kidney (3 cases), skin lesions (14 cases) and synovial membranes of the knee-joint (5 cases).

3.4. Molecular Hybridization

Molecular hybridization experiments were performed with ^{3}H-labeled measles virus RNA and DNA from various tissues of lupus erythematosus patients. Cellular DNA was prepared from i) blood leukocytes from three patients with lupus hepatitis, ii) the cell sediment of urine from a patient with lupus nephritis and iii) lymph nodes, bone marrow, kidney and spleen from two fatal cases of generalized lupus erythematosus.

Figure 6 presents the results of measles RNA hybridization with DNA from leukocytes (Fig. 6 A) or from lymph nodes (Fig. 6 B) of lupus erythematosus patients. In all three cases $\frac{1}{2}$ Cot values exceed 1000 mol. sec/l, that correspond to hybridization of unique sequences (one viral genome equivalent per cell). The results of hybridization with DNA from bone-marrow or cell sediment of urine of lupus erythematosus patients are shown in Figure 7 A and B, respectively. In these cases $\frac{1}{2}$ Cot values of 500 mol. sec/l and 240 mol. sec/l, respectively, were obtained which correspond to hybridization of reiterated sequences (2 to 5 viral genomes equivalent per cell) (35, 36). No hybridization of viral RNA with DNA from normal human tissues was observed, even at Cot values corresponding to 3000—5000 mol. sec/l, resultant from the heterogeneity of tissue systems used for virus propagation *in vitro* (CEF, Vero cells) and cellular DNA preparation (human tissues).

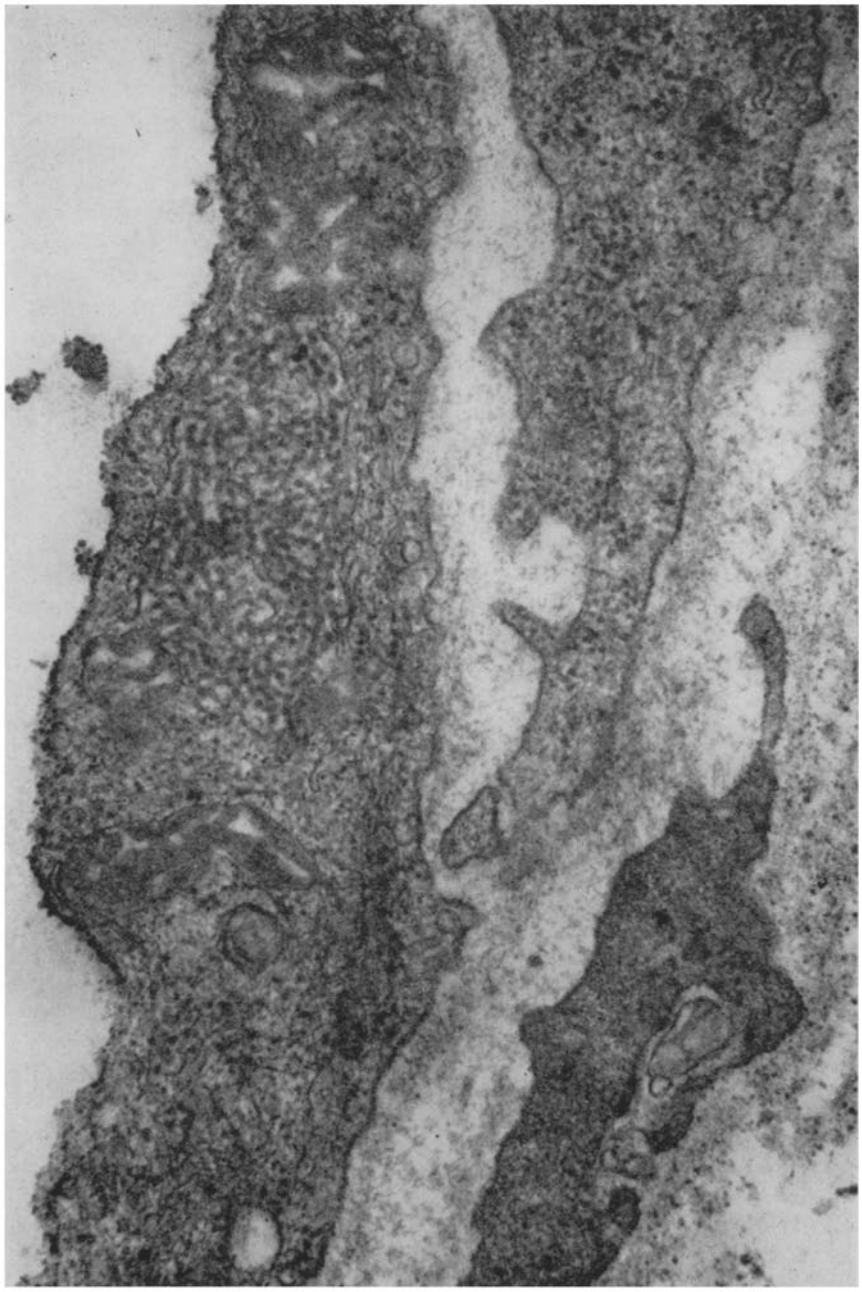

Fig. 2. Cross-section of a capillary in the synovial membrane of the knee-joint from a lupus erythematosus patient Tubular structures in a cytoplasmic inclusion of an endothelial cell are seen. Ultrathin section, magn. 15,000×

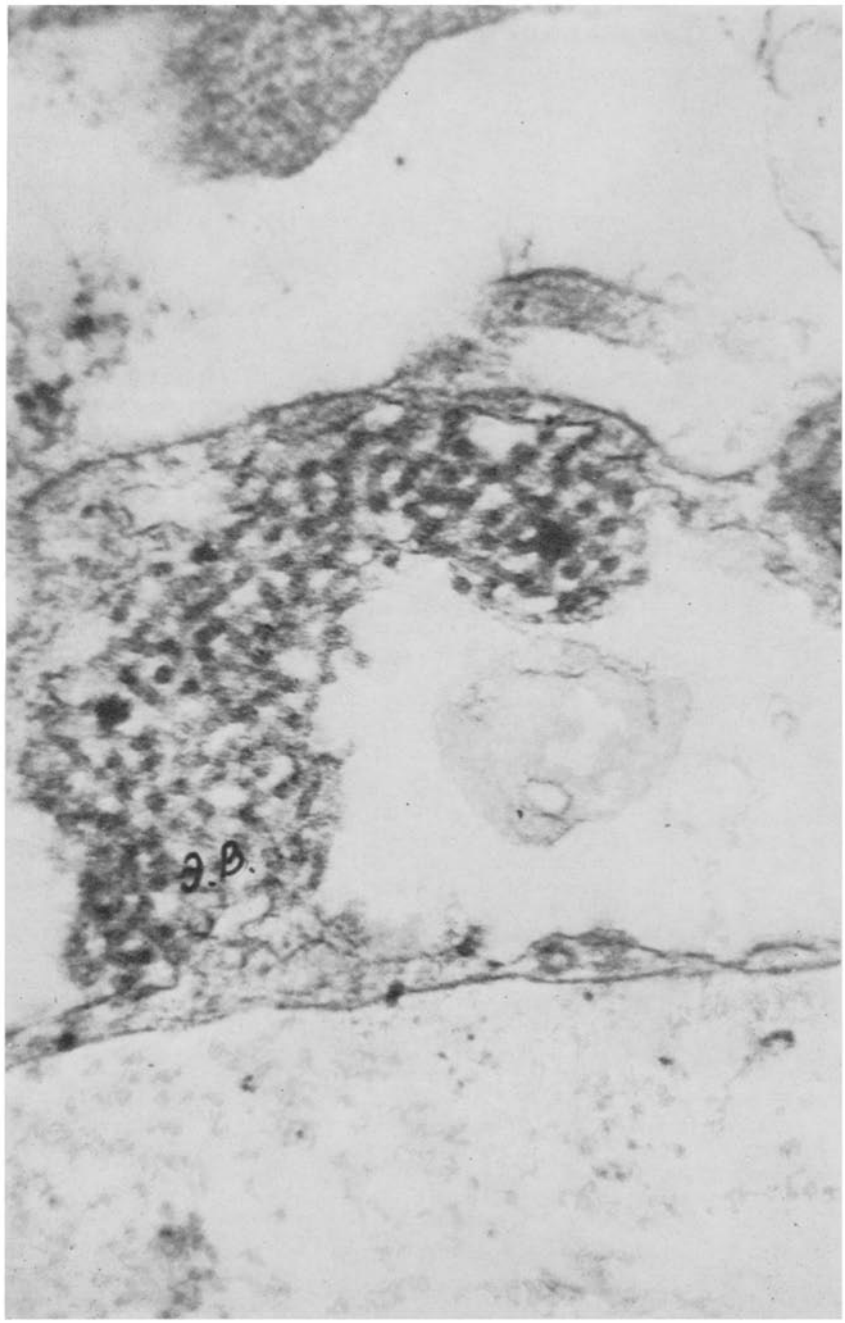

Fig. 3. Cross-section of an endothelial cell of a capillary of the kidney from a lupus erythematosus patient. Tubular structures are seen. Ultrathin section, magn. 40,000×

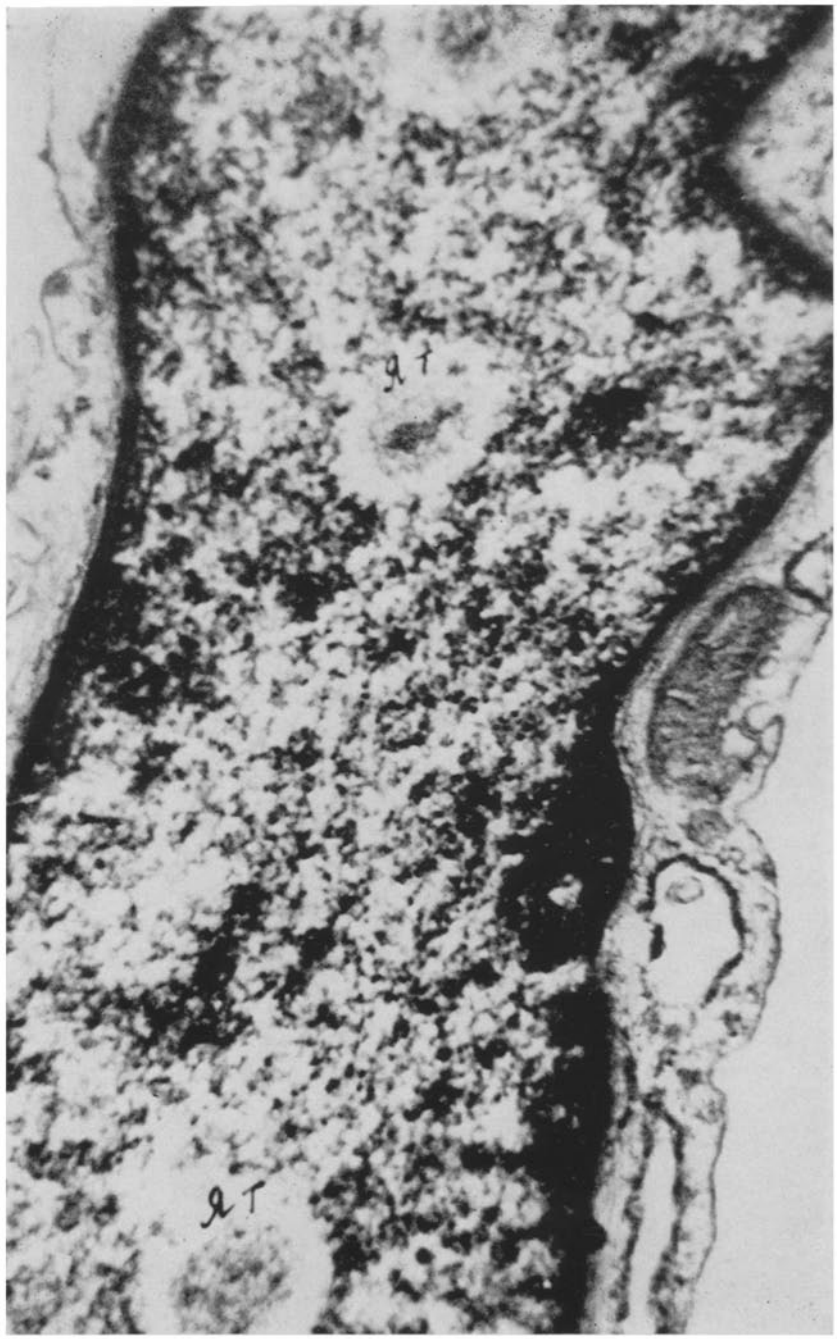

Fig. 4. Cross-section of the nucleus of a fibroblast of the skin from a lupus erythematosus patient
Nuclear inclusions are seen. Ultrathin section, magn. 50,000×

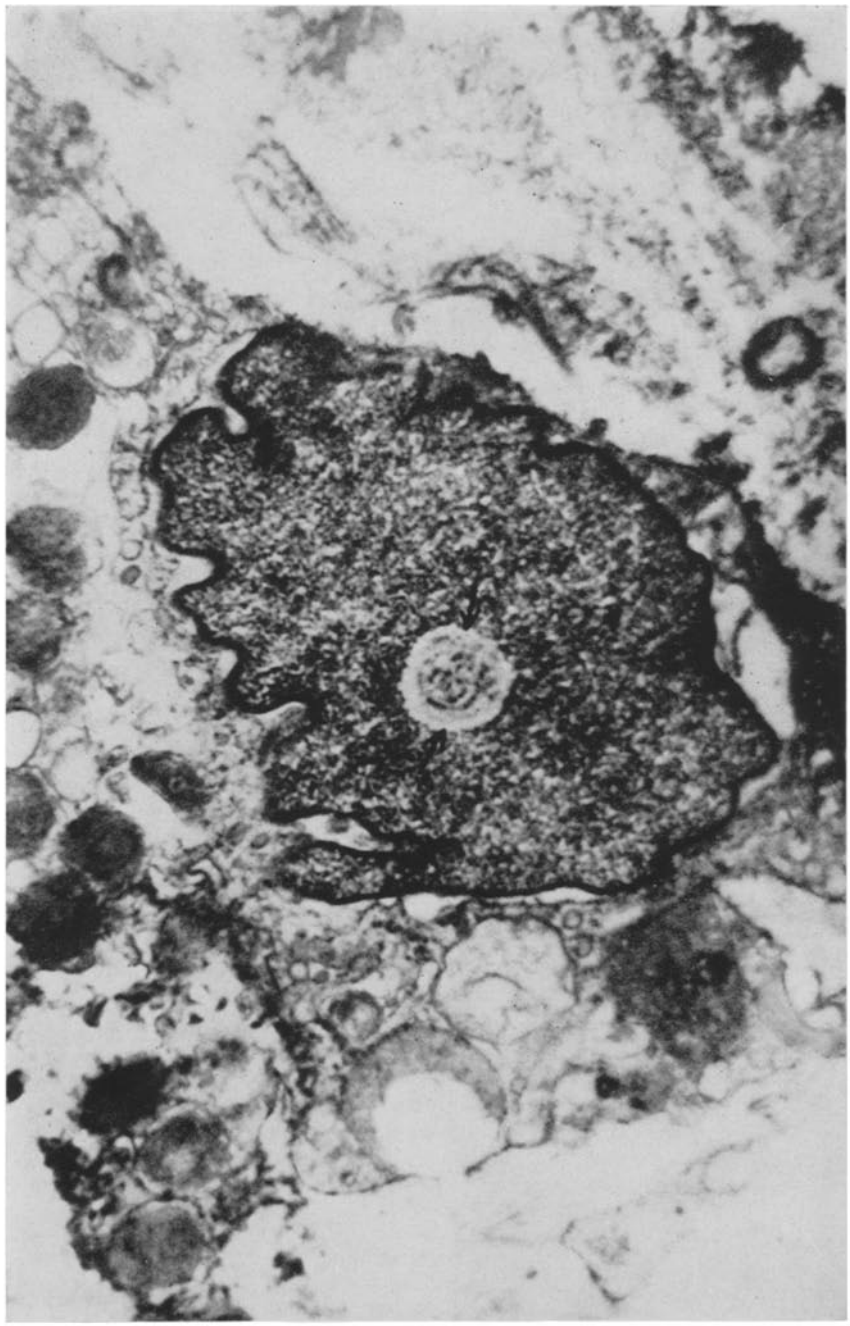

Fig. 5. Cross-section of the nucleus of a synovial cell of the knee-joint from a lupus erythematosus patient
Nuclear inclusions with tubular structures (arrow) are seen. Ultrathin section, magn. 20,000×

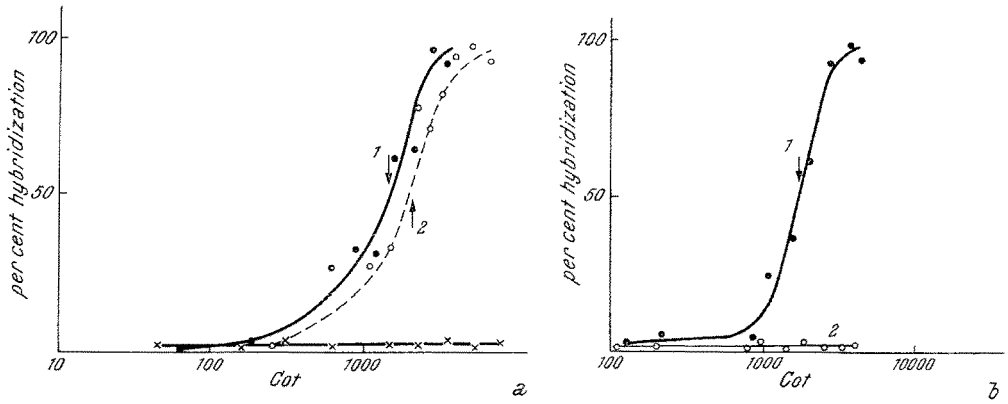

Fig. 6. Kinetics of hybridization (normalized curves) of ^{3}H-labeled measles virus RNA with DNA from leukocytes (A) and the lymph node (B) of lupus erythematosus patients
A. 1, 2 DNA from leukocytes of two lupus patients, 3 DNA from normal human placenta;
B. 1-DNA from a lymph node of a fatal lupus case, 2-DNA from normal spleen

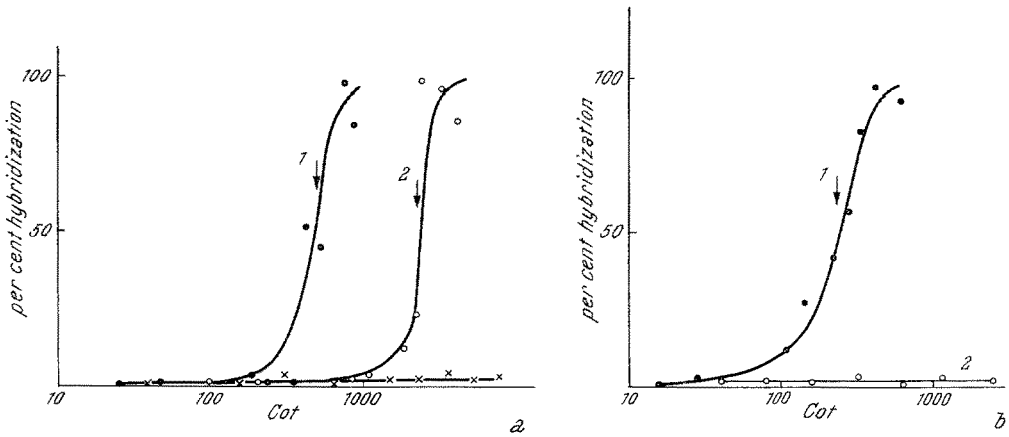

Fig. 7. Kinetics of hybridization (normalized curves) of ^{3}H-labeled measles virus RNA with DNA from bone-marrow (A), the cell sediment from urine (B) of lymphoma patients
A. 1-DNA from bone marrow of a lymphoma patient, 2-DNA from normal human placenta;
B. 1-DNA from the cell sediment of a fatal lupus nephritis case, 2-DNA from normal human placenta

4. Discussion

The results presented in this study strongly suggest that systemic lupus erythematosus, a typical autoimmune disease, might be caused by measles virus or another closely related paramyxovirus. This assumption is based both on the extraordinary high measles antibody titre in the blood of lupus patients and the detection of paramyxovirus-like structures (microtubules) in various lupus-affected tissues. These findings already reported by other investigators (11—16;

1—10) have been confirmed. Further evidence for the measles virus etiology of the disease—or at least the persistence of measles virus in patients with lupus erythematosus—has been supplied by the detection of measles virus antigen in immunofluorescence tests. Yet, the main proof of the etiological role of measles virus in lupus erythematosus was furnished by hybridization assays performed with viral RNA and cellular DNA. In these experiments it could be clearly demonstrated that the measles virus genome (DNA transcript) is integrated into nuclear DNA, thus becoming a constituent of the cell genome. This integration is expressed in the synthesis of virus-specific RNA and proteins which, however, are rarely or never completed to mature virions. This lack may be explained by the fact that measles virus belongs to the so-called "negative-strand RNA viruses", whose genome is transcribed into "positive RNA strands" which code virus specific proteins. Therefore, the formation of mature virions might be only possible if a well-balanced transcription of both strands of measles virus DNA sequences would occur for being intregated into cellular DNA. This event seems to be very rare and may account for the unsuccessful attempts to isolate virus from lupus-affected tissues.

However, a partial transcription of measles virus DNA sequences may be a less rare event and may result in the synthesis of various virus-specific proteins. Thus, the formation of tubular structures resembling viral nucleocapsids may be the expression of genes coding the nucleoprotein (S-antigen) of the virion. Other genes may code viral envelope proteins which, once incorporated into the cell membrane, modify the antigenic composition of the latter in such a way that the production of antibody (both against virus and cellular components) is stimulated. Moreover, cells with antigenically transformed cellular surface become targets for the attack of immunocompetent lymphoid-T cells. This is, to our mind, the chain of events which initiate autoimmune reactions including the formation of antibody against nucleic acids in the course of lupus erythematosus and some other autoimmune diseases (27, 37, 38).

The mechanism of transcription of measles virus RNA into DNA and integration of DNA transcripts into the cell genome remains to be elucidated. Previous experiments with tissue cultures chronically infected with measles virus (34) or tick-borne encephalitis virus (39, 40) demonstrated that latent oncornaviruses might help to accomplish reverse transcription. Whether such a cooperation of viruses might occur also *in vivo* should be especially studied.

In the future, it would be worthwile to study, whether the pathogenesis of other autoimmune and chronic degenerative diseases might be due to similar mechanisms as those found in systemic lupus erythematosus.

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