Endothelial inclusions and 'nuclear bodies' in systemic lupus erythematosus

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In recent years many reports on special cytoplasmic inclusions in cells of involved tissues of various organs in a number of 'autoimmune' diseases have been published. Most frequently, these inclusions are demonstrated in the endothelial cells of blood vessels, and are therefore often called 'endothelial inclusions' (EI), though they are occasionally found in fibroblasts, pericytes, macrophages, lymphocytes, and plasma cells.

In systemic lupus erythematosus (SLE), EI have been described in the kidney (Fresco, 1968; Györkey, Min, Sincovics, and Györkey, 1969; Györkey and Györkey, 1971; Kawano, Miller, and Kimmelstiel, 1969; Hurd, Eigenbrodt, Ziff, and Strunk, 1969; Hurd, Eigenbrodt, Strunk, and Ziff, 1970; Sinkovics, Györkey, and Thoma, 1969; Pincus, Blacklow, Grimley, and Bellanti, 1970; Grausz, Earley, Stephens, Lee, and Hopper, 1970; Helder, Blomjous, Feltkamp-Vroom, and van Loghem, 1971), skeletal muscle (Norton, 1969), skin (Hashimoto, 1969; Helder and others 1971), and synovial tissue (Schumacher, 1970; Dreyer, Muldiyarov, Nassonova, and Alekberova, 1973).

It has been assumed that EI are related to the possible aetiological factor of SLE and that their tubular structures are either of virus origin or appear as a result of virus infection. The majority of investigators believe that these tubular structures closely resemble the nucleoprotein strands of paramyxoviruses.

Recently, Neumark and Farkas (1970) described so-called 'nuclear bodies' (NB) in rheumatoid (RA) synovial tissue and suggested that these inclusions were also of virus origin. These authors found NB in endothelial cells, pericytes, and lining cells of 22 patients, but failed to find them in control subjects.

We studied biopsies of synovial tissue, kidney, and skin in definite SLE and found both EI and NB in the same tissue samples. The NB in SLE proved to be even more characteristic of the affected synovial tissue than the EI.

Material and methods

Five synovial biopsies, three skin biopsies, and two kidney biopsies from nine patients with definite SLE were studied with the electron microscope. The skin biopsies were taken only from uninvolved areas. A brief case history and laboratory data of each patient are summarized in the Table. The specimens were fixed for 1 to 2 hrs in ice-cold 2.5 per cent. glutaraldehyde, buffered to pH 7.4 with phosphate buffer, washed for 30 min. in phosphate buffer, post-fixed in cold Caulfield's fixative for 2 hrs, dehydrated with ethanol, and embedded in araldite. The tissue blocks were stained with 1 per cent. uranyl acetate in 70 per cent. ethanol during dehydration.

Ultrathin sections were prepared on an LKB III ultramicrotome, stained with lead citrate (Reynolds, 1963), and examined in a JEM-7 electron microscope.

Sections 2 to 4 μ m. thick were made of all the tissue blocks embedded for electron microscopy, stained according to Richardson, Jarett, and Finke (1960), and examined in the light microscope for orientation.

Results

Characteristic EI were found in the cells of synovial blood vessels and of the peritubular and glomerular capillaries of kidney. Sometimes there were two EI in one endothelial cell.

The typical EI represented more or less dense clusters of undulating tubules measuring from 200 to 250 Å in external diameter. The number and packing density of the tubules in the EI varied considerably. The electron density of the tubules increased after staining *en bloc* with uranyl acetate during dehydration (Figs 1 and 2).

Most commonly, the EI were surrounded by a single smooth limiting membrane which could not always be clearly seen or was sometimes absent. Clusters of undulating tubules without limiting membranes were sometimes found in the perinuclear spaces and, more often, in the cisternae of the granular endoplasmic reticulum (Fig. 2). The EI were distributed in both the perinuclear and the attenuated peripheral endothelial cytoplasm. They were not

Table *Clinical history and laboratory data*

Patient No.	Age (yrs)	Sex	Duration of disease (yrs)	Clinical and laboratory manifestations						Biopsy	'Endo- thelial inclusions'	'Nuclear bodies'	Antibodies to*		Antibodies to viruses†		
				Disease activity	Serum CH ₅₀	Serum globulins (% of total serum protein)	ESR (mm./1st hr)	LE cells	DNA				RNA	Measles	Kubella	Influenza B	
1	26	F	1.5	Arthritis Adhesive pleurisy Pericarditis Cardiopathies	29	11.3	37.0	40	+	Synovial tissue	+	++	1:320	1:40	1:1,280	1:32	1:640
2	22	F	0.5	Arthralgia	35	13.7	20.8	48	—	Synovial tissue	+	++	1:20	1:40	1:20	1:64	1:320
3	22	F	1	Arthritis Myositis Adhesive pleurisy Cardiopathies	36	9.9	22.0	36	+	Synovial tissue	—	—	1:80	1:80	1:2,560	ND	1:320
4	34	F	10	Arthritis Myositis Pneumonitis Polyserositis	25	13.5	27.0	60	—	Synovial tissue	—	+	1:80	ND	1:80	1:32	1:40
5	22	F	0.5	Arthritis Polyserositis	37	13.5	28.0	38	+	(1) Synovial tissue (2) Skin	+	+	ND	ND	ND	ND	ND
6	19	F	1	Erythema Arthritis Myocarditis Vasculitis	ND	11.9	18.3	47	—	Skin	—	—	1:160	0	1:2,560	1:256	1:1,280
7	42	F	7	Arthritis Pneumofibrosis Cardiopathy	39	11.6	23.0	29	—	Skin	—	—	1:20	0	1:80	0	ND
8	23	F	11	Nephritis Pericarditis Pleurisy	<3	14.4	23.4	47	+	Kidney	++	+	1:80	1:20	1:160	1:64	1:1,280
9	34	F	7	Nephritis Polyserositis Arthralgia	34	—	—	18	—	Kidney	++	+	ND	ND	1:80	1:32	1:320

* haemagglutination test

† haemagglutination inhibition test

observed in the mesangial cells, fibroblasts, glomerular, and tubular epithelial cells of the kidney, in the synovial lining, or in the connective tissue cells.

In addition, we found NB in endothelial cells and pericytes. Not infrequently, they were shown in other cells, including synovial lining cells and tubular epithelial cells. The NB usually looked like tangles of undefined filaments with a round to oval osmiophobic area of up to 0.5 μm. diameter around them (Figs 3 and 4).

The NB had no limiting membranes. There were often several NB in one nucleus (Fig. 4), sometimes side-by-side with an intact nucleolus (Fig. 4).

In the cells with NB one could observe myelin figures, degenerative organelles, ruptures of the nuclear and/or plasma membranes, and disintegration of the cytoplasm, while, as a rule, there was

no evidence of degenerative changes in the cells containing EI. On the whole, EI were shown in three of five synovial biopsies and in both renal biopsies (Table). All these specimens also contained NB. It was striking that in synovial tissue there were more EI than NB, while the contrary pattern proved to be the case in renal biopsies. NB were also present in one synovial and one renal biopsy in which we failed to observe EI.

The absence of EI in the synovial tissue of Patients 3 and 4 may be accounted for by the fact that the specimens consisted mostly of avascular fibrous and/or adipose tissue. We were not successful in finding EI in biopsies from uninvolved skin, though Garancis, Komorowski, Bernhard, and Straumfjord (1971) observed them with the same frequency in both uninvolved and erythematous areas.

Most of our patients had raised titres of serum

FIG. 1 Case 8. *Kidney. Inclusion in endothelial cytoplasm of interstitial capillary. The limiting membrane (M) is not clearly visible throughout its length. L—lumen. $\times 14,000$*

FIG. 2 Case 5. *Synovial capillary. Inclusion within profile of endoplasmic reticulum. $\times 45,000$*

antibodies to DNA ($\geq 1:80$) and RNA (poly A: poly U) ($\geq 1:20$), and to the viruses of measles ($\geq 1:80$), influenza B ($\geq 1:80$), and rubella ($\geq 1:32$). In all

cases, when both EI and NB were observed in tissues by electron microscopy (Cases 1, 2, 8, 9), the titres of antiviral antibodies were increased (Table).

FIG. 3 Case 5. Nuclear body (arrow) in endothelial cell of synovial capillary. L—lumen. $\times 15,000$

FIG. 4 Case 5. Two nuclear bodies in B-type synovial lining cell. The mitochondria (M) are swollen, the profiles of endoplasmic reticulum are dilated. N—nucleolus. $\times 12,000$

Discussion

In spite of a great number of observations on EI, their origin remains obscure. Most investigators agree with the opinion that the EI in SLE and other autoimmune

diseases are related to virus infection. The undulating tubules are generally regarded as bearing a close resemblance to the nucleoprotein strands of the paramyxoviruses. They also assume that EI can represent either cytoplasmic products, the appearance

of which is due to peculiar viral infection (Norton, 1969), or cellular material released from injured cells and phagocytosed by endothelial cells (Hurd and others, 1969). In some way or other, virus infection can thus serve as a stress, triggering an 'autoimmune' process.

As far as NB are concerned, Neumark and Farkas (1970), who observed them in the RA synovium, supposed that they also were connected with possible virus infection.

Our observations indicate that NB are not structures specific for RA. Moreover, in lupus synovitis, they occurred in considerably greater numbers than in RA synovitis. In a section of a nucleus one could often observe three or more NB. In other respects, these nuclei appeared to be intact. The NB represented the tangles of undefined filaments with an electron lucent zone around them approximately 0.5 μ m. in diameter. They had no limiting membranes, but there were clear-cut boundaries between the NB and surrounding chromatin. We did not observe NB either as they left the nuclei, or as intermediate forms as between EI and NB.

In SLE there were both EI and NB in the same synovial and renal biopsies. In the synovial tissue NB occurred substantially more often than EI, but in the renal biopsies one could see an inverse relationship. Considering that synovitis is an earlier clinical manifestation of SLE than nephritis, it may be assumed that NB provided an earlier submicroscopic evidence of the disease than EI. According to our data the range of distribution of NB is greater than that of EI. The latter are found almost exclusively in the vascular endothelial cells that may be associated with the systemic vasculitis so characteristic of SLE. Another salient feature of SLE is the appearance of serum antinuclear factors. This may have relevance to the development of NB. It should be noted that antinuclear antibodies occur in RA, even if more rarely and in lower titres than in SLE (Walton, 1971). With reference to this point, Neumark and Farkas (1970) revealed NB in RA synovium in very small numbers.

Norton (1969) failed to elicit myxovirus antigens in tissues containing EI. At the same time, it is known that the level of serum antibodies to the viruses of measles, rubella, influenza, parainfluenza, and mumps is significantly raised in SLE (Phillips and Christian, 1970; Hollinger, Sharp, Lidsky, and Rawls,

1971; Nasonova, Alekberova, and Vasilieva, 1972). Most of our patients had increased titres of antibodies to DNA and RNA; all the patients studied had increased titres of antibodies to measles, influenza B, and rubella viruses. If three patients, from whom the uninvolved skin biopsies were obtained, are not taken into consideration, we have four patients out of the remaining six with raised titres of serum antiviral antibodies and, at the same time, EI and NB in their tissue samples.

These results do not yet indicate the virus origin of SLE, since increased titres of antiviral antibodies and EI have been also found in other 'autoimmune' diseases. Rather they demonstrate increased antibody responsiveness to a variety of virus antigens in these pathological conditions, including SLE (Hurd and others, 1970). Nevertheless, the mere fact of the simultaneous finding of EI and NB is noteworthy, because both are considered as possible evidence of virus infection. We believe that further studies on the nature of EI and NB must include the demonstration of virus antigens in these structures by electron microscopic and immunocytochemical methods.

Summary

Five synovial biopsies, three skin biopsies, and two kidney biopsies from nine patients with definite SLE were studied by electron microscopy. Both endothelial inclusions and nuclear bodies were found in three of the five synovial biopsies and both of the kidney biopsies. In the synovial tissue, NB occurred substantially more frequently than EI, but in the renal biopsies one could see an inverse ratio. The possible relationship of EI and NB to virus infection is discussed.

ADDENDUM

During the preparation of this paper for publication we have studied a biopsy from the involved area of the skin of a 54-year-old female patient with definite SLE, the duration of the disease being 27 yrs. The EI were found in the endothelial cells and pericytes; a great number of NB were encountered in the vascular endothelial cells and fibroblasts.

The titres of antibodies were: to RNA (poly A:poly U) 1:40; to DNA ND; to viruses of measles 1:320; of rubella 1:32; of influenza B 1:1,280.

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