Nuclear bodies in rheumatoid synovium

T. NEUMARK AND K. FARKAS

National Institute of Rheumatism and Physiotherapy, Budapest, Hungary

There have been several reports on the fine structure of the synovial membrane in rheumatoid arthritis. All have stressed the cytoplasmic dense bodies which characterize the type A lining cell, but none have mentioned intranuclear bodies (Barland, Novikoff, and Hamerman, 1969; Hirohata and Kabayashi, 1964; Norton, Lewis, and Ziff, 1966; Marin, Negoescu, Stoia, Pierette, Petrescu, and Constinescu, 1969). Our own studies, however, have revealed characteristic nuclear inclusions in rheumatoid synovial membrane. In the present paper we describe and illustrate these, and discuss their possible significance.

Material and methods

Synovial membrane was obtained from 22 patients with rheumatoid arthritis during synovectomy of the knee which was carried out under spinal anaesthesia. The ages of the patients ranged from 20 to 69 years. The disease had been present in all of them for several years. Seven were strongly positive for the latex-fixation test. Ten had been treated with prednisolone*, 15 to 45 mg./day for from 9 months to 5 years.

Control specimens were obtained from six non-rheumatoid patients who were undergoing meniscectomy, or were being operated on for fracture of the patella. Needle biopsies of the peristriate area of the right cerebral cortex of eight patients suffering from subacute sclerosing encephalitis, a condition known to be associated with nuclear inclusions containing the helical components of measles virus (Adels, Gajdusek, Gibbs, Albrecht, and Rogers, 1968; Waterson, 1965; Tellez-Nagel and Harter, 1966b) were also studied.

After excision, specimens were immediately flooded with buffered Karnovsky fluid at pH 7.2 (Karnovsky, 1965) and then immersed for 1 hour in buffered 1 per cent. osmium tetroxide. After dehydration in alcohol, specimens were embedded in Durcupan.† Sections were cut with a Reichert OM-2 ultramicrotome, stained with uranyl acetate and lead citrate according to the method of Reynolds (1963), and examined in a Philips EM-300 electron microscope.

Results

Characteristic nuclear bodies were found in all the rheumatoid specimens but in none of the control specimens of synovial membranes. They were mostly located in the endothelial cells and pericytes of capillaries and/or larger vessels beneath the synovocyte lining layers and in deeper zones. They were also seen less frequently in cells judged to be mononuclear cells. Although ten blocks were taken from every specimen, generally only five showed intranuclear inclusions. In many cases multiple sections were needed to detect the presence of nuclear bodies. However, it was not possible to correlate the number of intranuclear bodies with any of the various clinical findings. Nuclear bodies were not seen in Type A or Type B synoviocytes, lymphocytes, or plasma cells.

The most common type of nuclear body is illustrated in Figs 1a, 1c, 2a, 2c, and 3a. It consists essentially of groups of filamentous or tubulo-filamentous structures, often surrounded by a clear halo. In individual sections of a nucleus one or more of these bodies might be seen. Their diameter varied from 0.4 to 1.6 μ . Variations in structure were noted which were partly due to the plane of sections. In Fig. 1a most of the filaments are cut longitudinally but a small group can be seen in cross-section. In some instances the nuclear body was covered by a thin shell of radially-arranged filaments (Fig. 1b). In others the whole body was made up of concentrically arranged striated filaments about 80 to 100 Å in diameter (Fig. 2c). Occasionally the inclusion appeared to lie inside a dilated nuclear membrane. Another type of nuclear body, sometimes found in the same cell as the tubulo-filamentous bodies, consisted of electron dense particles about 800 Å in diameter surrounded by a less electron dense shell (Fig. 4b). A third type of nuclear body was composed of close-packed dense particles, about 80 Å in diameter, forming an irregular mosaic-like structure (Fig. 4a). A fourth type of nuclear body had a complex structure consisting of a core of dense

*G. Richter, Budapest X, Hungary.

†Durcupan ACM, Fluke AG, Chemische Fabrik 9470, Bucks SG, Switzerland.

FIG. 1a Part of a fibroblast nucleus. Nuclear body (outlined) contains filamentous structures in longitudinal and (arrow) in cross-section. $\times 27,000$.

FIG. 1b Nuclear body (outlined) bounded by radially-arranged filaments. $\times 54,000$.

FIG. 1c Typical nuclear inclusion with surrounding clear halo (outlined). ESP = extracellular space. $\times 10,000$.

granular material surrounded by a shell of filamentous elements (Fig. 6). Electron dense particles 600 to 1,200 Å in diameter bearing a strong resemblance to those found by Gonatas (Gonatas and Shy, 1965; Gonatas, 1966; Gonatas, Martin, and Evagelista, 1967) were also seen, mainly in the nuclei but sometimes in the cytoplasm.

In two of the 22 rheumatoid patients a characteristic crystalloid formation was seen within the nuclei of connective tissue cells lying below the surface layers (Fig. 5). In serial sections these crystalloids were seen to be made up of a highly organized three-dimensional filamentous lattice lying in the long axis of the nucleus. The filaments of the lattice ran in parallel arrays at an included angle of 60° and showed an axial periodicity of 160 Å.

Neither the incidence nor the appearance of the nuclear bodies was correlated with clinical state,

treatment, or duration of the disease.

In addition to the nuclear bodies described above, the various abnormalities recorded by previous workers on the fine structure of rheumatoid synovium were also seen.

FIG. 2a Nuclear bodies in endothelial cells (arrow) $\times 10,500$.

FIG. 2b Nuclear body containing an eccentrically-placed dense particle (arrow). $\times 36,000$.

FIG. 2c Nuclear body (arrow) constructed of concentrically-arranged tubulo-filamentous elements 80-100 Å in diameter which show a cross-striation. $\times 54,000$.

FIG. 3a Various types of nuclear inclusion (arrows), one of which (upper left) has a granular core.

FIG. 3b Nuclear body (arrow) composed of electron dense granules surrounded by tubulo-filamentous structures. $\times 54,000$.

FIG. 4a Typical nuclear body lying in dilated part of nuclear membrane (arrow). Broken circle outlines a mosaic-like nuclear body composed of closely-packed particles measuring about $80 \text{ \AA}$ in diameter. Hexagon outlines inclusions; some show a central dense core and a less dense outer shell. $\times 37,000$.

FIG. 4b Tubulo-filamentous bodies (arrows) within a nucleus. Hexagons outline inclusions about $800 \text{ \AA}$ in diameter with a dense core and a less dense shell. aNm = aggregated nuclear material. $\times 16,000$.

FIG. 4c Part of a macrophage. Filamentous elements (arrow) $80\text{--}100 \text{ \AA}$ in diameter in longitudinal section. Ly = lysosome. $\times 22,000$.

Discussion

The present study indicates that nuclear bodies commonly occur in rheumatoid synovial membrane where they are concentrated in cells in and around small blood vessels and less frequently in fibroblasts and mononuclear cells. They did not occur in synoviocytes, lymphocytes, or plasma cells. These

inclusion bodies appear to be built up of filamentous structures about 80 to $100 \text{ \AA}$ in diameter. The manner in which the filaments aggregate and the plane of section may be responsible, at least in part, for the varied structural appearances encountered (Bouteille, Kalifat, and Delarue, 1967; Krishan, Uzman, and Hedley-Whyte, 1967).

FIG. 5 Characteristic intranuclear crystalloid in fibroblast. $\times 27,000$.

FIG. 6 Complex body (arrow) in a fibroblast. $\times 45,000$.

Nuclear bodies may occur in normal tissues or after treatment with ACTH (Horstmann, 1962, 1965; Horstman, Richter, and Roosen-Runge, 1966; Latta and Maunsbach, 1962a,b; Nicander, 1964; Weber, Whipp, Usenik, and Fromnes, 1964). As mentioned above, only ten of the 22 patients had received prednisolone, and none of the controls had received prednisolone. Nuclear bodies mostly occur in viral infections. The nuclear bodies illustrated in the present paper may be viral in origin, but we believe that the present structural evidence, though highly suggestive, does not allow us to conclude that the bodies are unquestionably virus particles or virus components. On the other hand, nuclear bodies closely similar to those we have found in rheumatoid synovium have been found in many known human virus diseases. These include the 'slow virus diseases' group (Gajdusek, Gibbs, and Alpers, 1965):

(a) Subacute sclerosing encephalitis (Escourolle, Berger, and Poirier, 1961; Périer and Vanderhaegen, 1966; Périer, Vanderhaegen, and Pelc, 1967; Ulrich and Kidd, 1966; Tellez-Nagel and Harter, 1966a; Dayan, Gostling, Greaves, Stevens, and Woodhouse, 1967; Shaw, Buchan, and Carlson, 1967; Herdon and Rubenstein, 1968; Ováry, Gombi, Benkö,

Gödény, Sorszegi, and Kappéter, 1968, 1969; Zu Rhein and Chou, 1968; Toga, Dubois, Berard, Tripiet, Cesarini, and Choux, 1969);

(b) Necrotizing encephalitis (Bouteille and others, 1967);

Cytomegalic inclusion disease (Krishan and others, 1967);

(c) Japanese B encephalitis, rabies, herpes simplex encephalitis (Bogaert, 1957; Hagmaker, Smith, Bogaert, and Chenar, 1958; Bergouignan, Julien, and Bital, 1967; Chou and Cherry, 1967).

Furthermore, crystalloid formations of the type found in two of our cases, though not specifically indicative of a virus, have been observed after polyoma and measles virus infection (Baker, Gordon, and Rapp, 1960; Anzai and Yoshikatsu, 1969), and the less structured electron dense bodies seen in some nuclei could conceivably represent the product of an interaction between incomplete virus and the host (Rowe, 1967).

On the basis of the present observations it seems important to search for additional structural evidence of the viral nature of the nuclear bodies. Secondly, if these bodies are associated with altered nuclear

metabolism, it should be possible to detect this, and we are currently engaged in studies to test this possibility.

Summary

Synovial membrane removed from 22 rheumatoid patients and control specimens from non-arthritic patients have been studied under the electron microscope. In all the rheumatoid specimens, but in none of the controls, characteristic nuclear bodies approximately 0.4 to 1.6 μ in diameter were detected, mainly in endothelial cells, perivascular connective tissue cells, and macrophages. The appearance of the bodies varied, but all the structural forms encountered closely resembled those seen in known viral infections. Further structural evidence will be needed before the bodies can be positively identified as virus or of viral origin.

The authors wish to express their sincere gratitude to Dr. L. Simon, chief of the Department of Orthopaedics of our Institute, for surgical material, and Dr. E. Óváry, Neuropsychiatric Clinic, School of Medicine, Budapest, for material obtained from the patients with subacute sclerosing panencephalitis.

References

- ADELS, B. R., GAJDUSEK, D. C., GIBBS, C. J., JR., ALBRECHT, P., AND ROGERS, N. G. (1968) *Neurology (Minneapolis)*, **18**, no. 1, pt 2, p. 30 (Attempts to transmit subacute sclerosing panencephalitis and isolate a measles related agent, with a study of the immune response in patients and experimental animals).
- ANZAI, T., AND OZAKI, Y. (1969) *Exp. molec. Path.*, **10**, 176 (Intranuclear crystal formation of poliovirus: electron microscopic observations).
- BAKER, R. F., GORDON, I., AND RAPP, F. (1960) *Nature (Lond.)*, **185**, 790 (Electron dense crystallites in nuclei of human amnion cells infected with measles virus).
- BARLAND, P., NOVIKOFF, A. B., AND HAMERMAN, D. (1969) *Amer. J. Path.*, **44**, 853 (Fine structure and cytochemistry of the rheumatoid synovial membrane, with special reference to lysosomes).
- BERGOUIGNAN, M., JULIEN, J., AND VITAL, C. (1967) *Rev. Neurol.*, **117**, 589 (Les encéphalites nécrisantes 'aiguës' de l'adulte, mise en évidence du virus herpétique au microscope électronique).
- BOGAERT, L. VAN (1957) 'Encéphalites d'origine inconnue', in 'Handbuch der speziellen pathologischen Anatomie und Histologie', ed. O. Lubarsch, F. Henke, and R. Rössle, band 13 'Nervensystem', ed. W. Scholz, teil 2, bandteil A, p. 394. Springer, Berlin.
- BOUTEILLE, M., KALIFAT, S. R., AND DELARUE, J. (1967) *J. Ultrastr. Res.*, **19**, 474 (Ultrastructural variations of nuclear bodies in human diseases).
- CHOU, S. M., AND CHERRY, J. D. (1967) *Neurology (Minneapolis)*, **17**, 575 (Ultrastructure of Cowdry type A inclusions. I. In human herpes simplex encephalitis).
- DAYAN, A. D., GOSTLING, J. V. T., GREAVES, J. L., STEVENS, D. W., AND WOODHOUSE, M. A. (1967) *Lancet*, **1**, 980 (Evidence of a pseudomyxovirus in the brain in subacute sclerosing leucoencephalitis).
- ESCOUROLLE, R., BERGER, B., AND POIRER, J. (1969) *Rev. française Neurol.*, **6**, 175 (Étude en microscopie électronique des inclusions intranucléaires dans trois cas de leucoencéphalite sclérosante subaiguë).
- GAJDUSEK, D. C., GIBBS, C. J., AND ALPERS, M. (1965) 'Slow Latent and Temperate Virus Infections'. NINDS Monograph No. 2.
- GONATAS, N. K. (1966) *J. Neuropath. exp. Neurol.*, **25**, 177 (Subacute sclerosing leucoencephalitis: electron-microscopic and cytochemical observations on a cerebral biopsy).
- , MARTIN, J., AND EVANGELISTA, I. (1967) *Ibid.*, **26**, 369 (The osmiophilic particles of astrocytes: viruses, lipid droplets or products of secretion?).
- AND SHY, G. M. (1965) *Nature (Lond.)*, **208**, 1338 (Virus-like particles in sub-acute sclerosing encephalitis).
- HAGMAKER, W., SMITH, M. G., BOGAERT, L. VAN, AND CHÉNAR, C. DE (1958) 'Pathology of Viral Diseases in Man Characterized by Nuclear Inclusions', in 'Viral Encephalitis'. Thomas, Springfield, Ill.
- HERDON, R. M., AND RUBINSTEIN, L. J. (1968) *Neurology (Minneapolis)*, **18**, no. 1, pt 2, p. 8 (Light and electron microscopy observation on the development of viral particles in the inclusions of Dawson's encephalitis (subacute sclerosing panencephalitis)).

- HIROHATA, K., AND KOBAYASHI, I. (1964) *Kobe J. med. Sci.*, **10**, 195 (Fine structures of the synovial tissue in rheumatoid arthritis).
- HORSTMANN, E. (1962) *Z. Zellforsch.*, **57**, 692 (Elektronenmikroskopie des menschlichen Nebenhodenepithels).
- (1965) *Ibid.*, **65**, 770 (Die Kerneinschlüsse im Nebenhodenepithel des Hundes).
- , RICHTER, R., AND ROOSEN-RUNGE, E. (1966) *Ibid.*, **69**, 69 (Zur elektronenmikroskopie der Kerneinschlüsse im menschlichen Nebenhodenepithel).
- KARNOVSKY, M. J. (1965) *J. Cell Biol.*, **27**, 137A (A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy).
- KRISHAN, A., UZMAN, B. G., AND HEDLEY-WHYTE, E. T. (1967) *J. Ultrastruct. Res.*, **19**, 563 (Nuclear bodies: a component of cell nuclei in hamster tissues and human tumors).
- LATTA, H., AND MAUNSBACH, A. B. (1962a) *Ibid.*, **6**, 547 (The juxtaglomerular apparatus as studied electron microscopically).
- , — (1962b) *Ibid.*, **6**, 562 (Relation of the centrolobular region of the glomerulus to the juxtaglomerular apparatus).
- MARIN, D., NEGOCESCU, M., STOIA, I., PIERETTE, A., PETRESCU, A., AND CONSTANTINESCU, S. (1969) *Acta rheum. scand.*, **15**, 126 (The morphology of the synovial tissue and articular fluid cells in rheumatoid polyarthritis—studied with the optical and electronic microscope).
- NICANDER, L. (1964) *Exp. Cell. Res.*, **34**, 533 (Fine structure and cytochemistry of nuclear inclusions in the dog epididymis).
- NORTON, W. L., LEWIS, D., AND ZIFF, M. (1966) *Arthr. and Rheum.*, **9**, 747 (Light and electron microscopic observations on the synovitis of Reiter's disease).
- ÓVÁRY, E., GOMBI, R., BENKŐ, K., GÖDÉNY, S., SORSZEGI, P., AND KAPPÉTER, I. (1968) *Ideggyógyászati Szemle (Hung.)*, **21**, 554 (Recent investigations in panencephalitis).
- , —, —, SORSZEGI, P., GÖDÉNY, S., AND KAPPÉTER, I. (1969) *Ibid.*, **22**, 165 (Data to the submicroscopical pathology of panencephalitis).
- PÉRIER, O., AND VANDERHAEGEN, J. I. (1966) *Rev. Neurol.*, **115**, 250 (Indications étiologiques apportées par la microscopie électronique dans certaines encéphalites humaines).
- , —, AND PELC, S. (1967) *Acta neuropath. (Berl.)*, **8**, 326 (Subacute sclerosing leuco-encephalitis. Electron microscopic finding in two cases with inclusion bodies).
- REYNOLDS, E. S. (1963) *J. Cell Biol.*, **17**, 208 (The use of lead citrate at high pH as an electron opaque stain in electron microscopy).
- ROWE, W. P. (1967) 'Some interactions of defective animals viruses', in 'The Gustav Stern Symposium. Perspectives in Virology V. Virus-directed Host Response', ed. M. Pollard, p. 123. Academic Press, London.
- SHAW, C.-M., BUCHAN, G. C., AND CARLSON, C. B. (1967) *New Engl. J. Med.*, **277**, 511 (Myxovirus as possible etiologic agent in subacute inclusion body encephalitis).
- TELLEZ-NAGEL, I., AND HARTER, D. H. (1966a) *J. Neuropath. exp. Neurol.*, **25**, 560 (Subacute sclerosing leukoencephalitis. I. Clinico-pathological, electron microscopic and virological observations).
- , — (1966b) *Science*, **154**, 899 (Subacute sclerosing leukoencephalitis: ultrastructure of intranuclear and intracytoplasmic inclusions).
- TOGA, M., DUBOIS, D., BERARD, M., TRIPIER, M. F., CESARINI, J. P., AND CHOUX, R. (1969) *Acta neuropath.*, **14**, 1 (Étude ultrastructurale de quatre cas de leuco-encéphalite sclérosante subaiguë).
- ULRICH, J., AND KIDD, M. (1966) *Ibid.*, **6**, 359 (Subacute inclusion body encephalitis. A histological and electron microscopical study).
- WATERSON, A. P. (1965) *Arch. ges. Virusforsch.*, **16**, 57 (Measles virus).
- WEBER, A., WHIPP, S., USENIK, E., AND FROMMES, S. (1964) *J. Ultrastruct. Res.*, **11**, 564 (Structural changes in the nuclear body in the adrenal zona fasciculata of the calf following the administration of ACTH).
- ZU RHEIN, G. M., AND CHOU, S. M. (1968) *Neurology (Minneap.)*, **18**, no. 1, pt 2, p. 146 (Subacute sclerosing panencephalitis. Ultrastructural study of a brain biopsy).