

Frequency and diagnostic value of the virus-like filamentous intranuclear inclusions in giant cell tumor of bone, not associated with paget's disease

A study of 43 cases

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Summary. This paper deals with the paramyxovirus-like intranuclear inclusions observed in giant cell tumours of bone (GCTB). Twenty-one (49%) of 43 cases of GCTB (1977–1985), either fresh and/or cultured, show these ultrastructural inclusions.

Fifty samples of various bone lesions in which giant cell lesions occurred, including aneurysmal cysts, hyperparathyroidism, osteoblastoma, human and rat osteopetrosis, GCT of tendon sheaths, and non skeletal granuloma were used as controls. These, together with 20 samples of normal bone (osteoclasts) did not contain intranuclear or intracytoplasmic viral inclusions.

Key words: Giant-cell – Virus-like inclusion – Intranuclear inclusion – Giant cell tumour of bone – Paget's disease of bone – Electron microscopy

Introduction

Rebel et al. (1974) described virus-like filamentous intranuclear inclusions which are considered to be characteristic of Paget's disease of bone (PDB). They are a constant feature in electron microscopic examination of Pagetoid bone and had been observed, four years previously, by Welsh and Mayer (1970) in the nuclei of the giant cells of two giant cell tumours of bone (GCTB), not associated with Paget's disease.

In 1977, we reported our first positive GCTB and we noticed that such intranuclear inclusions were apparently a rare ultrastructural feature, since they were not mentioned by several authors who

had done extensive ultrastructural studies of GCTB (Hanaoka et al. 1971; Kuwana 1967; Thiery et al. 1975). Two years later Aparisi et al. (1979) reported two further positive cases out of 11 GCTB examined.

We have studied 43 GCTB ultrastructurally, none associated with PDB. Fifty various bone lesions in which giant cell formation occurs, and normal osteoclasts were used as controls in order to establish the frequency of occurrence and the diagnostic value of the virus-like filamentous intranuclear inclusions.

Material and methods

47 surgical samples were obtained after biopsy or surgery of giant cell tumours (43 patients) not associated with Paget's disease and were classified according to Jaffe's criteria. Fresh samples were immediately prepared either for transmission electron microscopy (TEM) or for cell culture according to routine techniques (Vacher-Lavenu et al. 1981). In a further step, cell cultures were also prepared for TEM by using 3 per cent glutaraldehyde and 2 per cent OsO_4 . Fresh and cultured tissues were embedded in Epon and ultrathin sections were cut with an Ultracut. After lead and uranyl staining they were examined on an EM 10 Zeiss electron microscope under 80 KV voltage.

As controls 50 surgical samples of various bone lesions and 20 of normal bone were prepared in the same way (Table 1).

Results

21 GCTB (49%) were demonstrated to contain virus-like filamentous intranuclear inclusions. Paget's disease lesions were all positive (100%). In contrast, in normal osteoclasts and in various control bone lesions, we failed to identify any virus-like intranuclear or cytoplasmic material (Table 1).

It is noteworthy that in four patients intranuclear inclusions were only identified in recurrent

Table 1. Virus-like filamentous intranuclear inclusions in GCTB giant cells and various bone lesions with giant cell formation and normal osteoclasts

Bone lesions	Patients	Positive cases	
		Number	%
GCTB	43	21	49
Paget's disease	5	5	100
Aneurysmal cysts	3	0	0
Hyperparathyroidism	1	0	0
Osteoblastoma	2	0	0
Chondroblastoma	4	0	0
Human osteopetrosis	1	0	0
Rat osteopetrosis	5	0	0
GCT of Tendon			
Sheaths (1 malignant case)	4	0	0
Non skeletal granuloma			
mandibular	2	0	0
Villonodular synovitis	21	0	0
Bone sarcoma complicating			
Paget's disease	1	0	0*
Osteomalacia associated			
with Paget's disease of			
the sacral bone	1	0	0*
Normal osteoclasts	20	0	0

* Intranuclear inclusions were only seen in Pagetic bone

Table 2. Presence of virus like filamentous I.N.I.^a in GCTB giant cells from biopsies or cultured material

Biopsy samples	Primary GCTB (43)	Recurrences (4)	Total 47
Patients	39	4	43
Positive biopsies	16 (16)	4 (4)	20
Negative biopsies	23 (27)	0	23
Positive culture	7 (7)	2 (2)	9
Negative culture	22 (24)	2 (2)	24
Positive biopsy, positive culture	6	2	8
Positive biopsy, negative culture	10	2	12
Negative biopsy, positive culture	1	—	1
Negative biopsy, negative culture	12	—	12

Numbers without brackets are related to patients, while that in brackets are related to the samples studied

^a I.N.I.: Intranuclear Inclusions

GCTB, while in another patient the GCTB contained intranuclear inclusions only in the giant cells of the cultured material as shown in Table 2, which gives the detailed results.

The ultrastructural pattern of the virus-like filamentous intranuclear inclusions was similar to that of Paget's disease inclusions and has been described elsewhere (Abelanet et al. 1982; Vacher-

Lavenu et al. 1981; Welsh and Mayer, 1970) (Fig. 1).

In GCTB the inclusions were generally observed in the nuclei of the giant cells and rarely in the cytoplasm. It was sometimes difficult to find them and a long search was necessary. In contrast, they were easily found in Paget's disease osteoclasts in which they were more frequently observed, occurring either in the nuclei or in the cytoplasm (Abelanet et al. 1982).

Discussion

GCTB mostly occurs in young adults, with a mean age of 33.8 years. Three patients in our series were in their teens; the youngest was a 17 year old female, while the oldest was 81.

The rate of virus-like intranuclear inclusions observed in GCTB is about 49%. This figure is probably an underestimate and may increase with the investigator's experience. For example, in our first report (Le Charpentier et al. 1977) only one of fourteen cases was shown to contain such inclusions (7%) but five years later 35 GCTB were studied and 16 positives cases found (38%). Now we find these intranuclear inclusions in every new case. Rarely, intranuclear inclusions are only detected in tissue culture and not in the biopsy specimen. In cultured giant cells of GCTB, the pattern of the virus-like filamentous intranuclear inclusions is always the same as that of giant cells in fresh tissue and is similar to that of paramyxovirus nucleocapsids (Gherardi et al. 1980; Mills et al. 1976; Howatson et al. 1982; Schajowicz et al. 1980; Schultz et al. 1979; Singer et al. 1977, 1983). Intranuclear inclusions are not surrounded by a membrane. On transverse section they are 12 to 15 nm in diameter and share a clear central area of 5 to 6 nm. The tubulo-filaments are arranged in paracrystalline pattern and separated by a luscant band of 15 nm. Their size differs from that of intermediate filaments and microtubules (Erlandson 1981). However, recently intranuclear tubulo-filaments in one GCTB case have been found to be surrounded by an invaginating nuclear membrane in some instances (El Labban 1984). Thus non-trained investigators may interpret elements of the cytoskeleton as true intranuclear inclusions.

To our knowledge, the virus-like material is always found in multinucleated cells and has never been observed in stromal mononucleated cells or endothelial cells. Nuclear size reduction and fragmentation, as described by Welsh and Mayer (1970) are not correlated with the frequency of the virus-like inclusions.

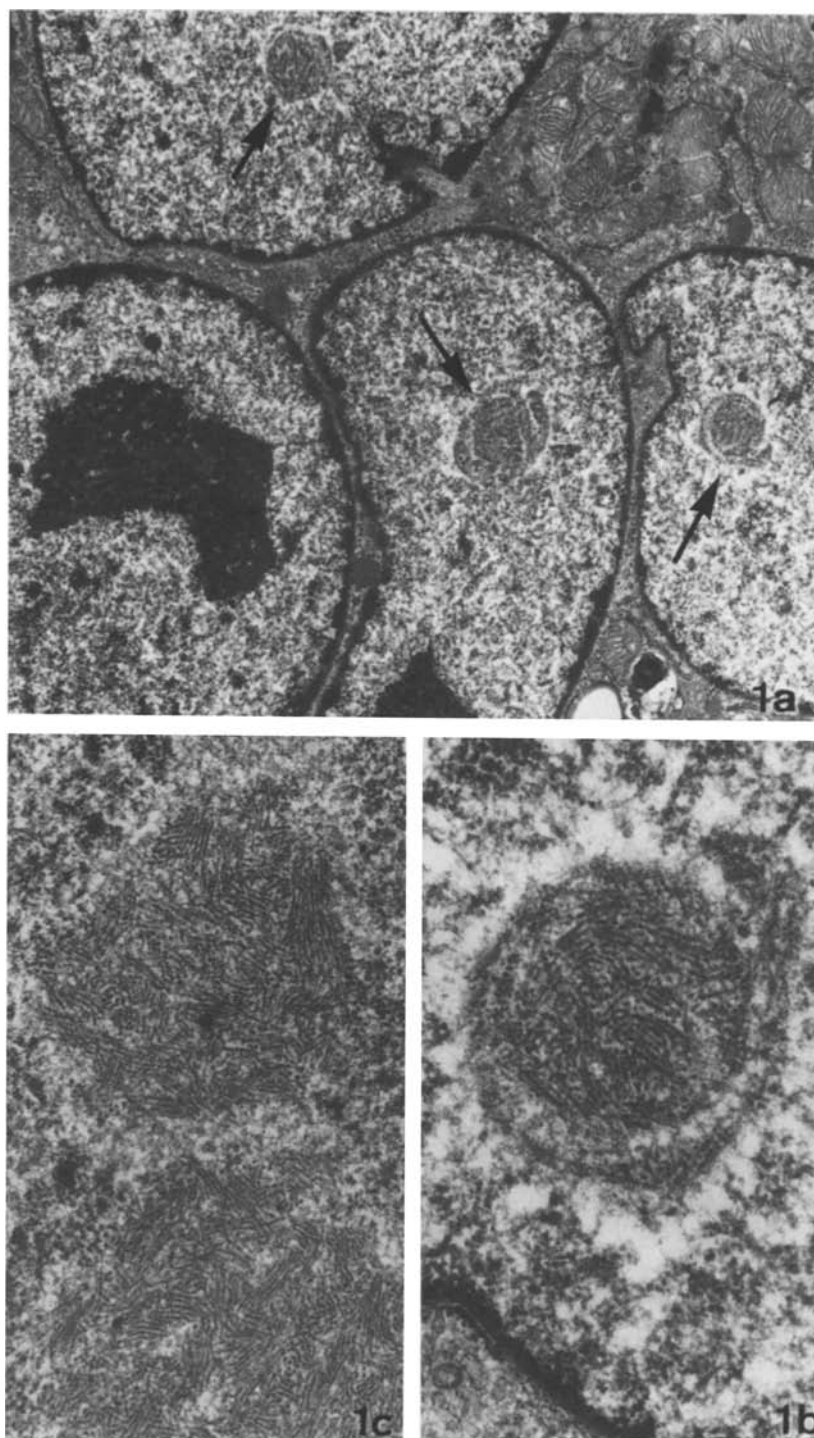


Fig. 1a. Cultured giant cell from GCTB. 3 nuclei contain typical tubulofilamentous inclusions (*arrows*) $\times 8,900$. **b** High magnification of an inclusion located on the right side of picture **a**, showing the tubulofilamentous structure $\times 42,900$. **c** The same pattern is observed in non cultured giant cells from a fresh biopsy of GCTB $\times 28,400$

Finding intranuclear inclusions is of marked diagnostic value in bone pathology. They are characteristic of Paget's disease and of GCTB; the only two bone diseases in which they may be found. In one patient their presence in a bone biopsy allowed a diagnosis of Paget's disease in spite of there being osteolytic lesions only. In another case

the previous diagnosis was aneurysmal bone cyst and here the identification of virus-like filamentous intranuclear inclusions allowed the diagnosis of GCTB.

The meaning of these inclusions is not clear although they are generally accepted as paramyxovirus-like. Rebel et al. (1974, 1980, 1981); Mills

et al. (1979, 1980); Singer et al. (1983) and other researchers (Gyorkey et al. 1971; Howatson et al. 1982; Morgan-Capner et al. 1981) who have done immunocytochemical studies on bone biopsies and cultured bone cells in Paget's disease have demonstrated positive response with antisera against the measles virus and/or respiratory syncytial virus. Based on these findings, the intranuclear inclusions have been suggested to be the expression either of measles or of respiratory syncytial virus. More recently, using a radiolabelled DNA probe, measles virus sequences were detected not only in multinucleated osteoclasts containing intranuclear inclusions but also in osteoblasts, osteocytes, endosteal cells and mononucleated cells, (Basle et al. 1986). These data supports the line of evidence that measles virus might be linked to Paget's disease of bone. In our opinion the aetiological role of the two viruses is still unproved. We are currently studying GCTB using immunocytological and in-site hybridization techniques.

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