

Infantile cortical hyperostosis (Caffey disease): ultrastructural and immunohistochemical characterization of the peritrabecular cells

Daniela Quacci¹, Ugo Ernesto Pazzaglia², Carlo Dell'Orbo¹ and Maria Grazia Ennas¹

¹Dipartimento di CitoMorfologia, Università degli Studi, Cagliari and ²Istituto di Clinica Ortopedica, Università degli Studi, Pavia, Italy

Summary. The ultrastructure and the immunohistochemical pattern of the cells which are responsible for the bone resorption in the cortical infantile hyperostosis were investigated.

The osteoclasts present a great positivity to MB1 antigen and a low positivity to OKM5. Mononuclear cells with primary lysosomes, looking like osteoclast ones are present in high concentration in peritrabecular spaces. These cells show a high positivity to OKM5 antigen and a low positivity to MB1 antigen. The mononuclear granulated cells are positive to tartrate-resistant acid phosphatase.

The possible common origin and their co-operation in bone resorption is discussed.

Key words: Infantile cortical hyperostosis, Ultrastructure, Immunohistochemistry

Introduction

Infantile cortical hyperostosis (ICH) is an uncommon self-limiting disease affecting children between the last month in utero and the sixth month of life, first described by Caffey in 1952. Affected children appear acutely ill, with high fever, irritability, painful swelling of the mandible and limbs, often symmetrically distributed (Saul et al., 1982).

In the less affected bones serial radiographs showed that abnormal undercalcified and not well organized bone trabeculae increase in amount and the original well organized ones are progressively removed out of existence (Pazzaglia et al., 1985).

The attempt of this paper is to describe the immunological pattern and the ultrastructure of the cells that are responsible for the enhanced bone resorption.

Materials and methods

Clinical case

The clinical aspects, radiological pictures and anatomo-pathological aspects of the case we have considered are described in previous papers (Beluffi et al., 1983; Pazzaglia et al., 1985).

Methods

Light microscopy

The fragments of various bones from autopsy were treated either by light microscopy, histoenzymological and immunological investigations or by electron microscopy investigations. The first ones were fixed in neutral-buffered 10% formalin, decalcified in 5% EDTA for a week, embedded in paraffin and sectioned 6 µm thick.

For morphological purposes deparaffinized sections were stained with haematoxylin and eosin or with Giemsa stain.

Tartrate-sensitive acid phosphatase

The deparaffinized sections were treated for 4 hours with a tartrated buffered solution (50 mM tartrate/0.2M acetate buffer pH 5.5) then the sections were incubated for 2 hours in a solution prepared by dissolving 25 mg naphthol phosphate in 2.5 ml dimethyl formamide with 12.5 ml of 0.2M Michaelis veronal acetate buffer and 32.5 ml distilled water. The solution was completed by adding 4 ml of a 1:1 mixture of pararosaniline solution (1 gr pararosaniline hydroxychloride in 20 ml distilled water and 5 ml concentrated hydroxychloric acid). The substrate solution was adjusted to pH 4.7 and filtered.

Immunology

The decalcified bone sections were deparaffinized and incubated in phenylhydrazine 0.1% in PBS for 30' in order to inhibit endogenous peroxidase activity. After washing

in 0.01 M phosphate-buffered saline (PBS) the slides were immersed in a 0.1% trypsin solution for 5'. After washing and incubation with normal goat serum (diluted 1/10 for 15') each section was incubated with primary antibodies for one hour. The reactivity and concentration of the antibodies are listed in Table I.

The slides were then rinsed three times in PBS, incubated for 30' with biotinylated antimouse IgG (1/50 Dako, Denmark) and subsequently with acetyl avidin biotinylated peroxidase complex (1/100 Bio-Division Italy) for 30'. A second inhibition of endogenous peroxidase was performed in Na-Azhide (1 mg/ml PBS) for 10'. The final brown staining was obtained by 10' incubation in 3,3' diaminobenzidine (3 mg/ml, SIGMA) and 0.3% H₂O₂ in PBS.

The tissue sections were then counterstained briefly with haematoxylin, dehydrated and mounted with polyvinyl alcohol solution.

Electron microscopy

Undecalcified specimen for electron microscopy were cut into small fragments and fixed in 3% glutaraldehyde dissolved in 0.1 M phosphate buffer pH 7.4, postfixed in 1% O₅O₄ dissolved in phosphate buffer, dehydrated in ethanol, treated with propylenoxide and embedded in Epoxy resin. Half micron thin sections were collected on slides and stained with 1% toluidine blue. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate.

Results

The cortical osteonic compact bone was almost completely absent and substituted by thin osseous trabeculae; inside them an osseous lamellar pattern or residual of osteonic structures were only rarely observable. Most of the trabeculae were made of primary bone, characterized by partial ossification and by the presence of osteoid material (Fig. 1). The lamellae were underlined by normal osteoblasts and by polynucleated osteoclasts.

The space between trabeculae was occupied by a richly vascular hypercellular connective tissue that appeared to be populated by a lot of mononuclear cells (Fig. 2). The prevalent family was represented by cells characterized by definite and regular borders and by a round, hyperchromic not lobated nucleus that was localized in the central cell space; the cytoplasm contained a lot of highly refrangent granules that are stained by eosin. Such cells were observed both in the peritrabecular and the perivascular side and inside the vessels.

On ultrastructural examination nuclear chromatin was condensed in a continuous line near the inner surface of nuclear cisternae and loose chromatin occupied the central part of the nucleus. Perinuclear cisterna was apparently continuous and only rarely presented nuclear pores. Relatively few cytoplasmic organules (tubules, filaments, free ribosomes) were observed and they were

usually present in perinuclear area. Mitochondria were scarcely represented. Rough reticulum was constituted by dilated cisternae containing a poorly electrondense material. On the other hand, cytoplasmic vesicles of intermediate and disomogeneous electronopacity, likely to be primary lysosomes, were evident. Golgi apparatus was evident in the perinuclear area (Fig. 3).

Adjacent to the free surface of the trabeculae, well-differentiated osteoclasts characteristically polynucleated were often evident (Fig. 4) and at electron microscopical observation they show a great number of lysosomes very similar to those described in mononuclear cells around the vessels and in the peritrabecular connective tissue (Fig. 5).

Tartrate resistant acid phosphatase reaction was strongly positive either in mononuclear cells with granular cytoplasm or in binucleated cells, probably a precursor of osteoclasts, and in osteoclasts that were closely fitted to osteoid matrix.

The membranes of the two above described cell types often joined up, suggesting cell fusion.

Mononuclear granulate cells presented a strong positivity to granulocyte, monocyte and macrophagic antibodies (OKM1, MAC 387, MT1, LCA). Particularly interesting was the positivity to typical antibody of monocytes OKM5 (Fig. 6).



Fig. 1. Compact diaphyseal bone is completely substituted by osteoid lamellae immersed in richly vascular tissue. x 10.

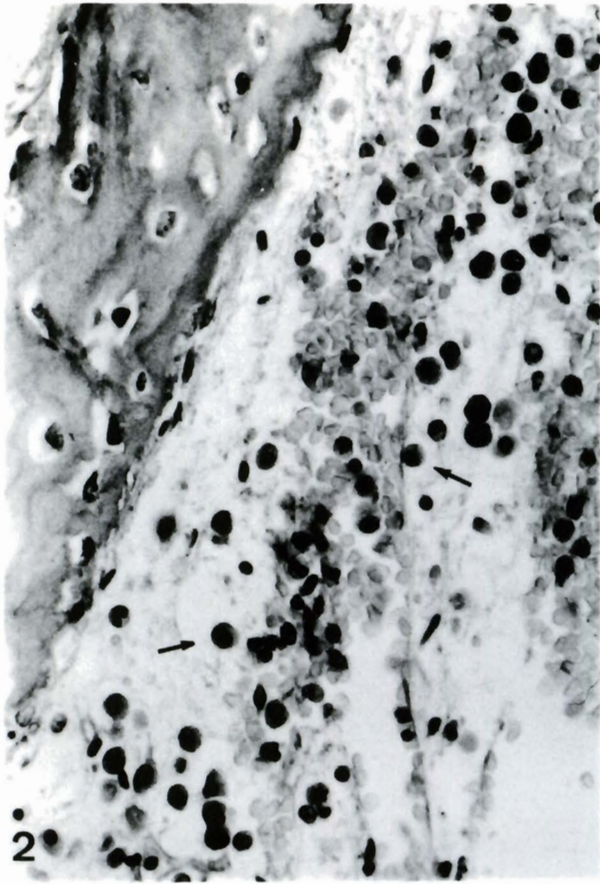


Fig. 2. The tissue in which lamellae are wrapped populated by a lot of mononucleated cells with a characteristic round nucleus and a strongly acidophilic cytoplasm (arrow) $\times 25$

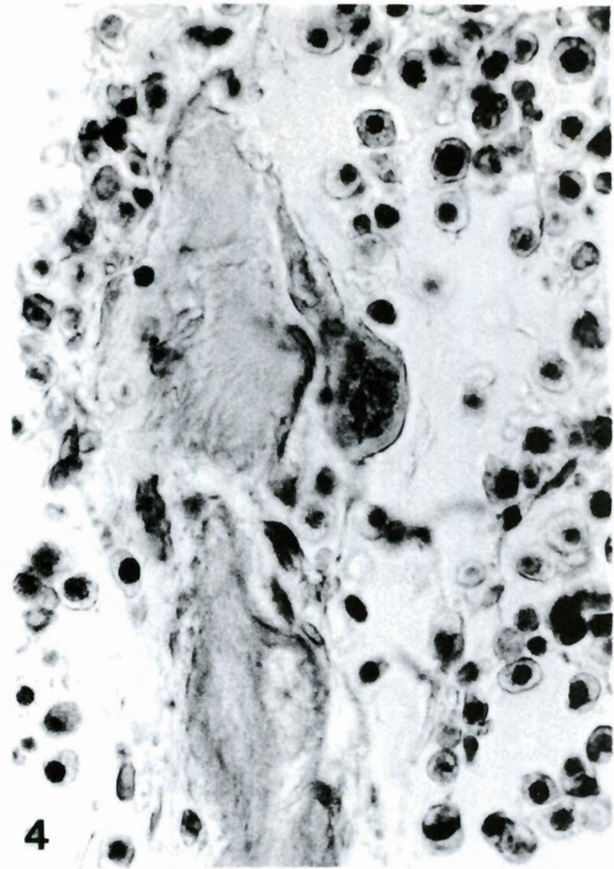


Fig. 4. On the surface of lamellae many polynucleated osteoclasts can be detected. $\times 40$

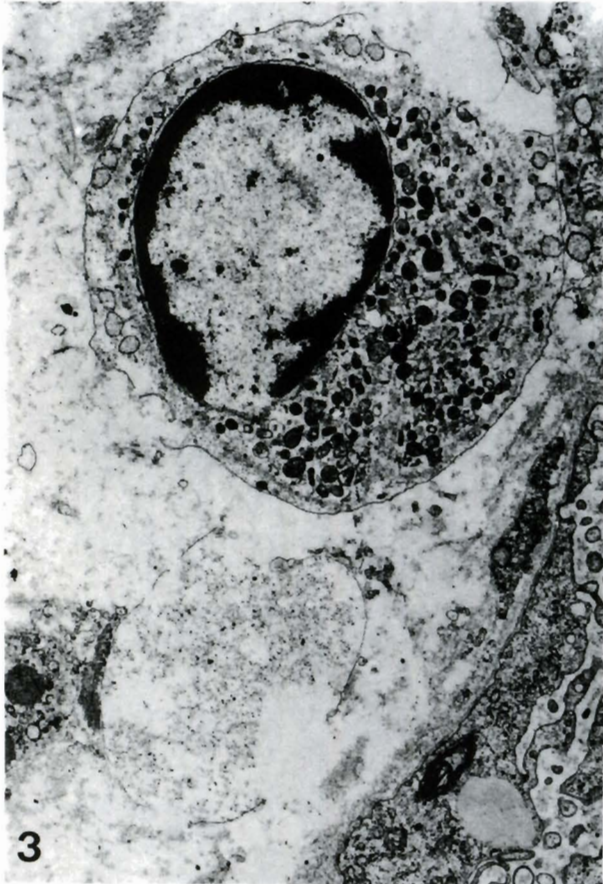


Fig. 3. At E.M. the mononuclear cells present a nucleus with margined chromatin and a cytoplasm showing a scarce amount of rough reticulum and a lot of heterogeneous electrondense vesicles. $\times 13,000$



Fig. 5. At E.M. level osteoclasts present the usual morphology: vesicles of intermediate electronopacity, some mitochondria and enlarged vesicles of rough reticulum. $\times 9,200$

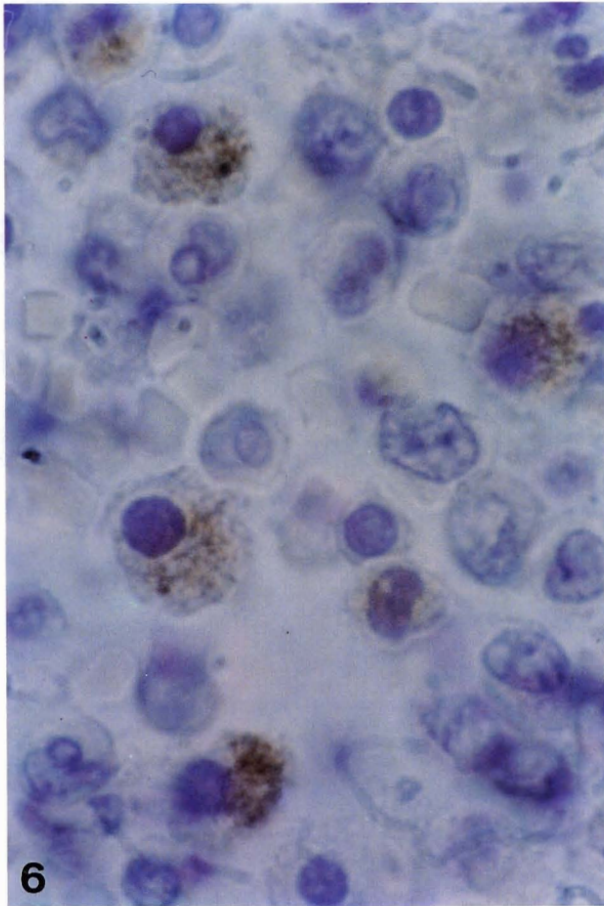


Fig. 6. OKM5 antibody-positivity is evident in mononucleated cells and sometimes in osteoclasts. $\times 100$

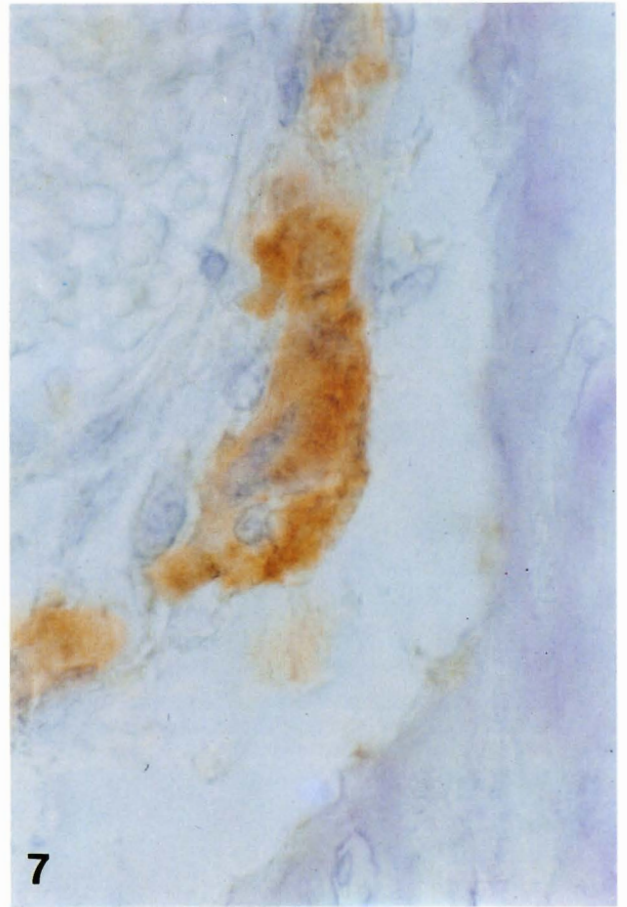


Fig. 7. MB1 antibody strongly stains osteoclasts underlying perivascular and peritrabecular space. $\times 100$

TABLE I

AB	Reactivity	References	Produced by	Concentration
MB1	B-Lymphocytes Plasmacells T-Mature Osteoclasts	Poppema et al., 1987 Chilosi et al., 1988	Biotest	(1/1)
OKM1	Granulocytes Monocytes Mielocytes (C3b)	Horton et al., 1984 Athanasou et al., 1986	Ortho	(1/5)
OKM5	Monocytes Platelets	Shen et al., 1983	Ortho	(1/5)
MAC387	Granulocytes Monocytes Istiocytes	Brandtzaeg et al., 1988	Dako	(1/40)
MT1	T-Lymphocytes Macrophages Granulocytes	Poppema et al., 1987	Biotest	(1/1)
LCA	Leucocytes Osteoclasts	Athanasou et al., 1987	Dako	(1/10)
HAM56	Macrophages	Gown et al., 1986	Ortho	(1/1)

TABLE II

	Mononucleated with granules	Binucleated with granules	Osteo- clasts	Other cells of bone marrow	Osteo- blasts
MB1	-	+	+++	-	-
OKM1	++ *	-	-	-	-
OKM5	+++	+	+	+	-
MAC 387	++	-	-	-	-
MT1	+++ **	+	-	-	-
LCA	+	-	-	+	-
HAM56	-	-	-	+	-
AC.PHOS	+++	+++	+++	-	-

(*).- Some cells are strongly positive, the others completely negative. (**).- Strongly positive in perivascular zones.

Binucleate granular cells, probably precursors of osteoclasts (light positivity to MB1), presented a diffuse positivity only to MT1 and OKM5 and MB1 antigen.

The osteoclasts were completely negative to all antigenic probes except to MB1, which showed a strong positivity (Fig. 7), and to OKM5, which showed a low positivity.

The osteoblasts that underline the trabeculae were completely negative to all antibodies tested.

HAM56 antigen was tested in order to evaluate the macrophagic antigen expressed on bone resorption cell surface and had a completely negative result.

On the other hand, MAC387 antigen, another macrophagic granulocytic antigen, was positive only on granulated mononuclear cells.

LCA (leucocyte common antigen) was poorly expressed in mononuclear granulated cells and in the other cells of bone marrow.

Immunological findings are resumed in Table II.

Discussion

Ultrastructural and immunological findings described were obtained by autoptic material, so the fixations were made about 20 hours after death and tissue and antigen preservation was not optimal. At the time of the autopsy there was not intention to make the immunological characterization of this tissue and for this reason the fragments were not preserved (cooled) in liquid air. In addition all fragments, embedded in paraffin were decalcified in EDTA. The antigen preservation was not optimal after aldehydic fixation, paraffin embedding and decalcification, and so the presence of some undetected antigens cannot be excluded, but also a low positivity can suppose a high antigen expression.

For immunological purposes, since the inhibition of endogenous peroxidase with methyl alcohol was incomplete, we applied the double inhibition of endogenous

peroxidase with phenyl-hydrazine and sodium-azide. The double inhibition was very satisfactory and the controls completely white.

The observed complete depletion of the cortical bone and, partially, of lamellar and osteonic bone, suggests a massive osteolysis in agreement with radiological findings (Pazzaglia et al., 1985). The number of osteoclasts present in the trabecular surface can justify the striking bone resorption. Within the perivascular connective tissue, near the trabeculae and often adherent to them, several mononuclear cells with cytoplasmic granules were evident. They showed a trend to confluence, were rich in lysosomes and positively stained with tartrate resistant acid phosphatase reaction. Some authors (Gahan, 1967; Chappard et al., 1983) think that acid phosphatase is not detectable after paraffin embedding: in our fragments a strong positivity was present after embedding and decalcification. This fact can evidence a high concentration and activity of such an enzyme. This character is considered sufficient by some authors to identify osteoclasts or osteoclast-like mononuclear cells (van de Wijngaert and Berger, 1986). Thus, similar cells were recognizable in normal animals as phagocytic mononuclear cells of blood origin (Ries, 1984). They started striking morphological and ultrastructural features on phagocytic activity, and may be considered responsible for bone resorption. Evidence was recently provided that also normal (physiological) bone remodelling in vivo (TranVan et al., 1982) and in vitro (Domínguez and Mundy, 1980) is due not only to osteoclastic activity on calcified matrix, likely following mononuclear prostaglandin activation, but also to direct action of the blood monocyctic-mononuclear phagocytes on the poorly or not calcified matrix (Jones et al., 1984).

The osteoclast cytoplasm was very similar to the mononuclear granular cell one: the granules have the same electronopacity, the same dimension and are arranged in the periphery of the cell. Also, cytoplasmic

organules were scarce in both cellular types. The osteoclasts were furthermore characterized by a strong positivity to MB1 osteoclast-specific antigen (Chilosi et al., 1988), but did not show any positivity to OKM1 antigen and only a little reactivity to OKM5, which were retained monocyte-specific antigens (Shen et al., 1983; Athanasou et al., 1986). The positivity to OKM5 was an unexpected result because in paraffin-embedded fragments this antigen is normally suppressed (Shen et al., 1983).

Mononuclear periosteal infiltration, elsewhere considered of lymphocytic origin (Pazzaglia et al., 1985), was deeply investigated in the present case. On both light microscopy and electron microscopy study, increased mononucleated cell rate with some evidence of cell differentiation from monocyte to mononuclear phagocytic cell was evident.

A lot of mononucleated cells of the perivascular and the peritrabecular connective tissue presented a high positivity to OKM5 antigen and also mononuclear cells with granular cytoplasm presented the same positivity.

Mononucleated granular cells expressed, even if at a low level, also granulocyte, lymphocyte and macrophagic antigens. It is well known that the loss of antigens expressed in immature cells is not an unusual aspect of blood and bone marrow evolution.

Binucleated cells expressed either tartrate resistant acid phosphatase activity or MB1 osteoclastic antigen or OKM5 and MT1 macrophagic antigen and may be considered an intermediate phase of osteoclast evolution (Ibbotson et al., 1984; Poppema et al., 1987).

Nevertheless, the morphological evidence can show a similar cytoplasmic aspect in granular mononuclear cells and osteoclasts, the expression of OKM5 (monocyte specific)-antigen was poorly detected in osteoclasts; on the contrary some mononuclear cells with granular cytoplasm presented an evident positivity to MB1 antigen and a high positivity to tartrate-resistant acid phosphatase.

Primary bone deposition, abundance of osteoid tissue and a large amount of active osteoblasts present within the tissues were easily recognizable as an attempt to maintain physical characters of bone tissue despite osteolysis.

In conclusion, it is not possible to detect the etiology of cortical infantile hyperostosis even if the infective inflammatory one is the more probable; it was not possible to demonstrate the sure appurtenance to the same cellular family of monocyte, of mononuclear cells with granular cytoplasm and osteoclasts: even if we consider the positivity of tartrate-resistant acid phosphatase MB1 antigen is completely lacking in mononuclear granulate cells and OKM5 has only a little positivity on osteoclasts. Ultrastructural evidence, however, suggests that all cell types considered work together for the bone resorption and it is possible to suspect that bone resorption is the first pathogenetic step of the bone disordered remodelling of infantile cortical hyperostosis.

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