06.81.34

Microspectrophotometric Quantitation of DNA in Bone Tumors with Giant Cells (Osteoclastoma, Osteosarcoma and Chondroblastoma)

F. SCHAJOWICZ, M.D., R. L. CABRINI, M.D., AND I. GIMENEZ, D.D.S.

Almost all authors agree that the genuine giant-cell tumor is an aggressive neoplastic process and that the origin of the mononuclear stromal cell is from the undifferentiated mesenchymal cell of the bone marrow. From these same stromal cells, with their tendency toward histiocytic and fibroblastic differentiation, derives the multinucleated giant cell. It develops either by fusion of the mononuclear stromal cells, as held by the majority of authors, or by repeated division of the nuclei of stromal cells without the corresponding cytoplasmic division, as suspected by Virchow and reiterated by Albertini.[1] One of us (F.S.), in collaboration with Lustig and Schajowicz[13] and Gallardo *et al.*,[4] has been able to prove that the latter mechanism occurs at least in some giant cells in tissue culture, probably by amitotic division or nuclear segmentation. Most ultrastructural studies lend more support to the fusion theory but have not been able to demonstrate it with certainty.[6,11,18,19]

One of us (F.S.)[16] has demonstrated that multinucleated giant cells show the same histochemical behavior as is observed in the normal osteoclast. These findings have been confirmed by other authors,[7,9,10,14] including evidence obtained from ultramicroscopic studies.[18] Identical histochemical and ultrastructural features characterize pathologic tumoral and nontumoral multinucleated cells. Giant cells of osteoclast type are also found in chondroblastoma and osteosarcoma. As a general rule, giant cells have a limited lifespan. Degenerative changes appear in giant cells in tissue cultures after 48 to 72 hours while the fundamental mononuclear stromal cells are still actively growing and proliferating. In contrast to the frequency of mitotic activity in the stromal cells, no mitotic figures, or only very few, have been demonstrated in the giant cells.[4] The mechanism of giant-cell formation is difficult to elucidate in static material, such as tissue samples, and this is also true in ultrastructural or tissue culture studies. To follow the growth and division of cells would require the use of microcinematography, a method not yet applied in the field of bone tumors.

In the last two decades DNA has been investigated microspectrophotometrically in many different neoplastic processes. According to these studies, the distribution of nuclear DNA is related to the cellular atypism, which is considered to be one of the indications of malignancy.[2,15] We have already published our findings in a series of chondromas and chondrosarcomas in which an evident correlation between histophoto-

From the Center of Osteoarticular Pathology (Italian Hospital) and the Department of Radiobiology (Comisión Nacional de Energía Atómica), Buenos Aires, Argentina.

Reprint requests to Dr. Schajowicz

Received: July 9, 1979.

0009-921X/81/0500/091 $00.85

metric results and the grade of malignancy was demonstrated.[17] In the present study we have applied this technique to bone tumors that contain a more or less conspicuous number of multinucleated giant cells, and have compared the quantity of DNA in the mononuclear stromal cells with that in the giant cells. We hope that this relatively new method may help to clarify some of the disputed aspects of the histogenesis and behavior of these tumors.

MATERIALS AND METHODS

We have studied six cases of osteoclastoma (giant cell tumor), eight cases of chondroblastoma (epiphyseal or benign chondroblastoma) and four cases of malignant bone-forming tumor (osteosarcoma). The osteosarcomas were selected cases showing abundant giant cells. One of them was a sarcomatous transformation in Paget's disease.

Biopsy material was fixed with formol and was embedded in paraffin. In order to perform the microspectrophotometric study of DNA, the Feulgen reaction was employed with 35 minutes hydrolysis. Sections were mounted on synthetic resin.

Quantitation was performed by means of a bidirectional scanning system and included about 120 plugs per nucleus. The equipment used was a Cytoscan Zeiss with automatic stage for scanning with an interval of 0.5 μm and a plug diameter of 0.5 μm.

In each tumor the DNA content of the giant cells and of the stromal cells surrounding them was determined. The lymphocytes in each tumor section were also measured to provide a normal diploid value and a system of comparison. Values for each tumor were recorded as histograms.

RESULTS

Osteoclastoma

Histograms corresponding to the six cases of osteoclastoma are represented in Figure 1. Distribution of the content of DNA in the lymphocytes is shown in the first column. The second column gives the results of the "individual" measurement of giant-cell nuclei. It is evident that the content of DNA in each of these giant-cell nuclei is very constant, almost without deviation, and markedly similar to the content of the lymphocytes. We can therefore affirm that the individual nuclei of giant-cells have a diploid content of DNA, that is to say, the same quantity of DNA as is found in all somatic cell nuclei. In the third column the histograms show the accumulated data for the stromal cells, of fibroblastic or histiocytic type. Although most nuclei have the diploid content, as do the nuclei of the giant cells, several values exceed it and give the graph an evident deviation to the right. Those cells with a larger DNA content than the diploid must be in a stage synthesis, which is indirect proof of their proliferative activity.

Figure 2 represents the accumulated data of all six osteoclastomas, and it clearly emphasizes the uniformity of the results obtained. It is again remarked that the diploid content of the nuclei of the giant cells indicates their lack of cellular reproduction. On the other hand, the deviation towards the right in the histogram for the stromal cells indicates cellular reproduction. These tumors must, therefore, proliferate at the expense of the stromal cells and not of the multinucleated cells which derive from them.

In none of these osteoclastomas was there evidence of irregular distribution of DNA which might suggest the presence of atypical cells.

Chondroblastoma (Epiphyseal or Benign Chondroblastoma)

Eight cases of chondroblastoma were analyzed (Fig. 3). The results are very similar to those recorded for the osteoclastomas, but this tumor offers a more complicated structure for this kind of study. The irregularity of its cellular elements and the foci of necrosis and calcification within it render difficult the selection of cells to be submitted to microspectrophotometric quantitation. For these reasons the histograms reveal some dispersion which is not biologically significant but merely the result of methodologic difficulties. The histograms show that in

chondroblastomas, as in osteoclastomas, giant-cell elements with multiple diploid nuclei occur, with no sign of right deviation. This suggests that such cells are without proliferative capability. Evidence of cellular proliferation of the stroma cells was found in the chondroblastomas as in the osteoblastomas.

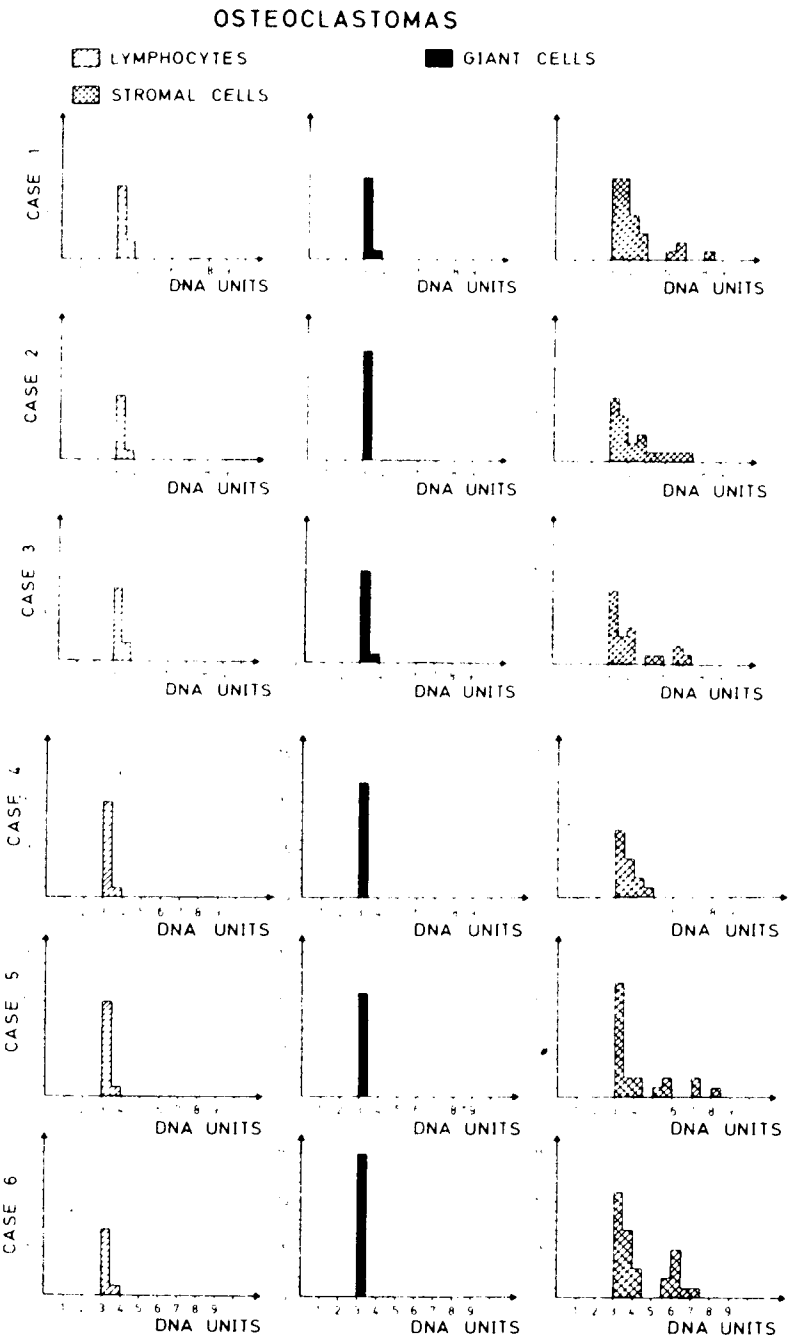

FIG. 1. Histograms corresponding to the six cases of osteoclastoma under study. The first column shows the distribution of DNA content in lymphocytes as a reference for normal diploid value. The second column shows results of individual measurement of giant-cell nuclei. The third column shows accumulated data for fibroblastic and histiocytic stromal cells.

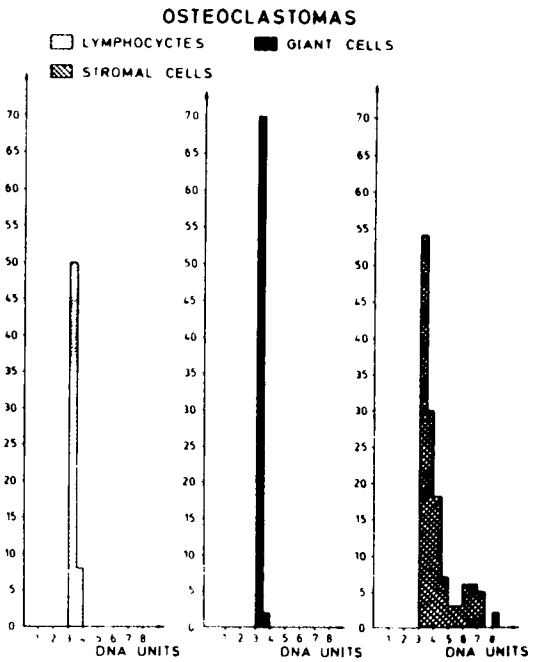

FIG. 2. Accumulated data collected from DNA–Feulgen measurements in six cases of osteoclastoma.

OSTEOSARCOMA

Four different types of cell were selected for study in osteosarcoma tumors. First, the lymphocyte was taken as the model of a somatic cell as before. The giant cells were divided into two groups, making types two and three: those of osteoclastic aspect with multiple regular nuclei, and atypical giant cells with few nuclei of very irregular or monstrous appearance. Fourth, were the cells of the tumoral stroma. The results of this study are shown in Figure 4.

The first group of giant cells had the diploid content in each individually analyzed nucleus. No deviation to the right was found and therefore no evidence of cellular reproduction existed. However, in the second group of giant-cells, the DNA content w[illegible] extremely variable, generally increased over the diploid content and with marked irregularities in its distribution.

The cells of the tumoral stroma produced a histogram concentrated in the diploid band but with frank deviation to right, indicating cellular proliferation. In these cells, too, the values of DNA were high and the irregular distribution suggested cellular atypism.

DISCUSSION

The microspectrophotometric data, as presented in this study, indicate the different

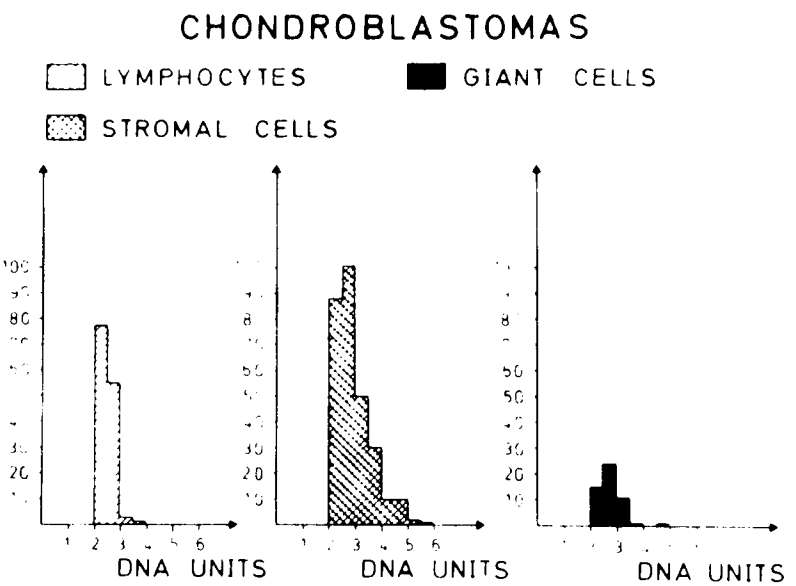

FIG. 3. Distribution of DNA content in eight cases of chondroblastoma. First column (lymphocytes) shows the normal diploid values.

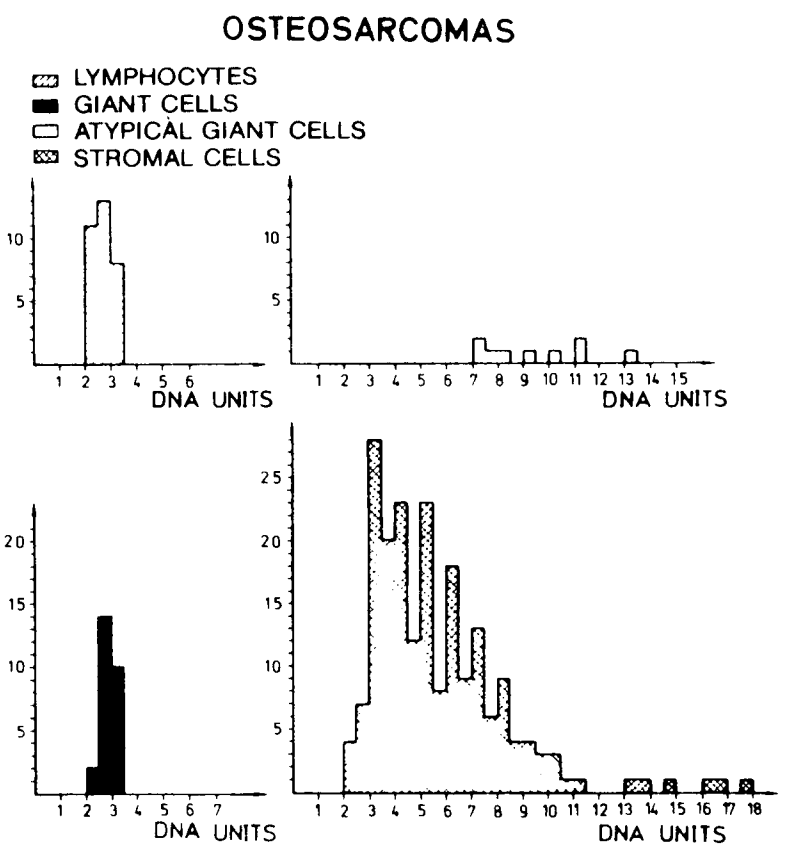

FIG. 4. Distribution of DNA content in four cases of malignant bone-forming tumors. Giant cells measured were divided in two groups: giant cells with normal histologic appearance and giant cells with atypical nuclei. Symbols: single hatch = lymphocytes, empty = atypical giant cells, black = giant cells with normal histologic appearance, double hatch = stromal cells.

behavior of the mononuclear cells of the stroma and the multinuclear giant cells, both in osteoclastoma (Fig. 5) and chondroblastoma.

The stromal cells are mostly diploid and their DNA distribution (asymmetry to the right) indicates active cellular proliferation. The giant cells in osteoclastomas and chondroblastomas also have nuclei with a diploid content of DNA. The uniformity of DNA content among the nuclei of giant cells speaks in favor of the existence of elements without mitotic activity. That their content should be so exactly diploid, and so similar to the model stroma cell, strongly suggests that giant cells have formed by conglomeration of stromal cells.

We do not deny the existence of other mechanisms of formation of giant cells, since mitotic division and gemmation have been observed in tissue culture. However, it should be emphasized that in tissue culture, cells may exhibit peculiar behavior and that the phenomenon known as "gigantization" is one such abnormal form. These methods of forming giant cells cannot be disregarded in tumors but the microspectrophotometric data indicate that they are exceptional.

Our findings confirm that in osteoclastoma and in chondroblastoma it is only the stromal cell that participates in active proliferation and that the giant cell is a by-product incapable of reproduction.

The analysis of the DNA content of osteosarcoma cells is also of interest. It was no surprise to find dispersion of the data in the stroma of these tumors. The giant cells fell into two groups: those which we can call "typical" of the osteoclastic type, and which characteristically had nuclei with a uniform

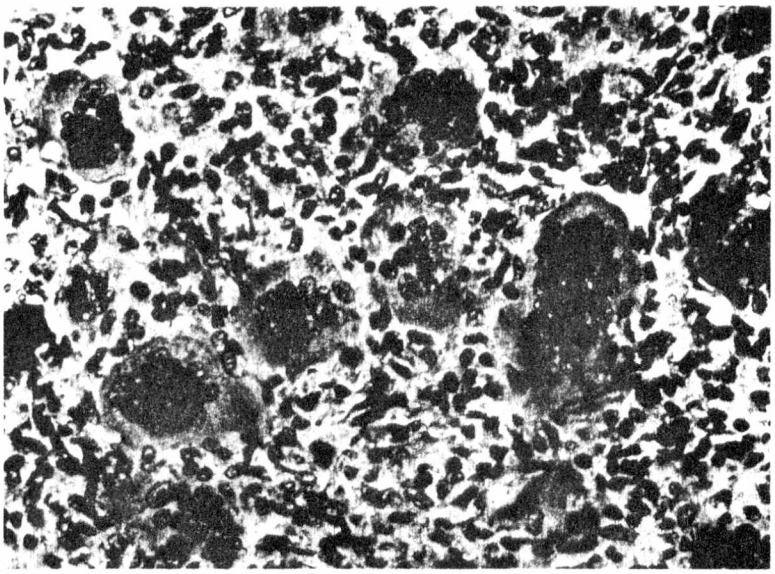

FIG. 5. Histologic appearance of one of the analyzed cases of osteoclastoma. (×220).

content of DNA; and those which were "atypical," with highly variable DNA content among the nuclei. It seems likely that the giant cells with diploid nuclei are not tumoral but result from the fusion of fibroblasts or histiocytes; whereas the second type of giant cell, which has an irregularly distributed DNA content, is a characteristic product of malignant growth.

SUMMARY

A microspectrophotometric study of DNA content was carried out on cells from three types of bone tumor—osteoclastoma, chondroblastoma, and osteosarcoma—in all of which the formation of multinucleated giant cells occurs. Giant cells of osteoclastic type were present in all the tumors, with contents of DNA which were constant, uniform and diploid in character for each nucleus individually considered. Since there were no nuclei with reduced or increased content of DNA, mitotic proliferation or gemmation can be excluded. Instead, the evidence suggests the formation of giant cells through a mechanism of cellular fusion. In contrast, the stromal cells of the osteoclastomas and the chondroblastomas gave evidence of DNA synthesis, thus proving their proliferative capacity in both tumors. The osteosarcomas showed a very irregular distribution of DNA, and generally the nuclear content of DNA exceeded the diploid DNA content.

REFERENCES

1. Albertini, A.: Gutartige Riesenzellengeschwüllste. Leipzig, Georg Thieme, 1928.
2. Atkin, N. B., Richards, B. M., and Ross, A. J.: The desoxyribonucleic acid contents of carcinoma of the uterus: An assessment of its possible significance in relation to histopathology and clinical course, based on data from 165 cases. Br. J. Cancer 13:173, 1959.
3. Dahlin, D. C.: Bone Tumors. General Aspects and an Analysis of Cases. Springfield, Charles C Thomas, 1978.
4. Gallardo, H., Lustig, E. S., and Schajowicz, F.: Growth and maintenance of human giant-cell bone tumor (osteoclastomas) in continuous culture. Oncology 24:146, 1970.
5. Goldenberg, R. R., Campbell, C. J., and Bonfiglio, M.: Giant-cell tumor of bone. An analysis of two hundred and eighteen cases. J. Bone Joint Surg. 52:619, 1970.
6. Hanoaka, H., Friedman, B., and Mack, R. P.: Ultrastructure and histogenesis of giant-cell tumor of bone. Cancer 25:1408, 1970.
7. Imura, S., and Takeda, M.: Histochemical and Electron Microscopic Studies on the Giant-Cell Tumors of Bone (Specially Cultured Cells). New York, Springer Verlag, 1968.
8. Jaffe, H. L.: Tumors and Tumorous Conditions of

the Bones and Joints. Philadelphia, Lea & Febiger, 1958.

9. Jeffree, G. M., and Price, C. H. G.: Bone tumors and their enzymes. A study of the phosphatases, non-specific esterases and betaglucuronidase of osteogenic and cartilaginous tumors, fibroblastic and giant-cell lesions. J. Bone Joint Surg. 47B:120, 1965.
10. Kraievsky, N. A., Raikhlin, N. T., and Soloviov, J. N.: Histochemical characteristics of giant-cell bone tumors. Folia Histochem. Cytochem. 8:3, 1970.
11. Kuwana, K.: Electron microscopic study on giant-cell tumor bone. Arch. Jpn. Chir. 36:819, 1967.
12. Lichtentstein, L.: Bone Tumors. St. Louis, The C. V. Mosby Co., 5th Ed., 1977.
13. Lustig, E. S., and Schajowicz, F.: Cultivo de tejidos de tumor giganto celular de los huesos. Contribución a la histogénesis de la célula gigante. Rev. Ortop. Traumatol. Latinoam. 14:134, 1944.
14. Pais, C., and Baruffaldi, O.: Ricerche istochemiche sui tumori a mieloplassi (contributo allo studio istogenetico del tumore e delle cellule giganti; significato ei reperti nella valutazione della malignita). Arch. Putti 10:9, 1958.
15. Sandritter, W., Carl, M., and Ritter, N.: Cytophotometric measurements of DNA contents of human malignant tumors by means of the Feulgen reaction. Acta Cytol. 10:26, 1966.
16. Schajowicz, F.: Giant-cell tumors of bone (osteoclastoma). A pathological and histiochemical study. J. Bone Joint Surg. 43A:1, 1961.
17. Schajowicz, F., Gimenez, L. B., Conti, C. J., and Cabrini, R. L.: Estudio microespectrofotométrico del ADN en condromas y condrosarcomas. Bol. Soc. Arg. Ortop. Traumatol. 2:147, 1976.
18. Steiner, G. C., Gosch, L., and Dorfman, H. D.: Ultrastructure of giant-cell tumors of bone. Hum. Pathol. 3:569, 1972.
19. Ueno, K.: Electron microscopic observations of giant-cell tumors of bone. J. Jpn. Orthop. Assn. 38:724, 1964.