DOES MYELOMA SECRETE AN OSTEOBLAST INHIBITING FACTOR?

CAROL E. EVANS, CHARLES S. B. GALASKO, CAROL WARD

From the Hope Hospital, Manchester

Unlike most other tumours, myeloma causes bone destruction without an osteoblastic reaction; we tried to assess whether myeloma secretes a humoral factor that inhibits osteoblasts. Human bone-derived cells were either co-cultured with myeloma cells, or cultured in medium conditioned by myeloma cells. Bone-derived cell growth was measured by cell counts and by uptake of tritiated thymidine (^{3}H-Tdr); growth was inhibited when cultured in medium conditioned by myeloma cells and some inhibition was seen when the bone-derived cells were co-cultured with myeloma cells. The inhibiting effect was dose-dependent and also dependent upon the density of the myeloma cells conditioning the medium. The results of our study suggest that myeloma secretes an osteoblast inhibiting factor of less than 50,000 Dalton molecular weight.

Bone destruction is usually associated with reactive new bone formation. This occurs with the majority of bone tumours, and with virtually all metastases with the exception of most of the myelomata, and many solid deposits from leukaemia or lymphoma. Irrespective of the radiographic appearance, histological examination of a metastasis usually shows a combination of bone destruction and formation. If the former predominates the lesion appears lytic, whereas if the latter predominates as in prostatic metastases, the lesion is sclerotic on radiographs (Galasko 1986).

Myeloma deposits are different. Radiographically the lesion is lytic, and histologically there are large numbers of osteoclasts with no reactive new bone formation and no osteoblastic response (Galasko 1986). We therefore investigated whether the absence of an osteoblastic response was due to the secretion of an osteoblast inhibitory factor by the myeloma cells.

PATIENTS, MATERIALS AND METHODS

Pieces of cancellous bone were taken at operation from patients who were having bone removed for other purposes, or from the femoral head when this was removed at arthroplasty. The fragments of bone, approximately 4 mm^2, were cultured in 5 ml of minimum essential medium (Eagle's Modification) (MEM). The bone-derived cells (BDC) which grew out from these samples were set up in 24 well plates at a seeding density of 1×10^4 per cm^2. Previous studies have shown that

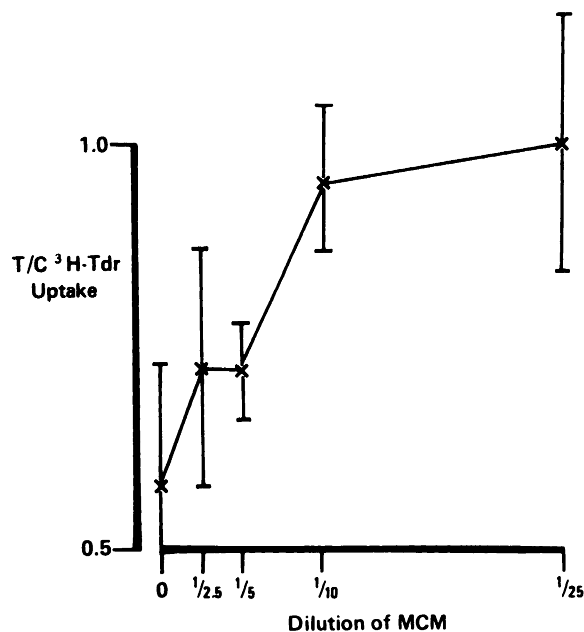

Fig. 1

Effect of the dilution of medium conditioned by myeloma cells on DNA synthesis in bone-derived cells. The points are the means of the test/control (T/C) ratio ± 1 s.d. for four patients. Assays for each patient were performed in triplicate.

C. E. Evans, BSc, PhD, ChBiol, Research Fellow
C. S. B. Galasko, MSc, ChM, FRCS, FRCS Ed, Professor of Orthopaedic Surgery
C. Ward, Research Technician
University of Manchester, Department of Orthopaedic Surgery, Hope Hospital, Eccles Old Road, Salford M6 8HD, England.

Correspondence should be sent to Dr C. E. Evans.

© 1989 British Editorial Society of Bone and Joint Surgery
0301-620X/89/2059 $2.00
J Bone Joint Surg [Br] 1989;71-B:288-90.

these cells behave as osteoblasts (Gallagher et al. 1983; Ashton et al. 1985; Evans, Galasko and Ward 1986). The BDC were cultured in medium conditioned by myeloma cells (MCM) over a range of dilutions (0 to 1/25), and also with fractions of the conditioned medium which had been obtained by filtration across a 50,000 Da cut-off membrane. In a further experiment, the BDC were cultured with viable myeloma cells at 0.5×10^4 per cm^2, or with a range of cell densities varying from 0.5×10^4 to 10×10^4. The myeloma cells had been cultured from an established cell line (GM.1500) obtained from the Wistar Institute and cultured in RPMI 1640.

BDC proliferation was assayed by cell counts before and after 48 hours incubation with medium or myeloma cells, and by the uptake of tritiated thymidine by the BDC after 48 hours incubation. The controls were BDC cultured in fresh RPMI or in medium previously conditioned by the bone-derived cells themselves.

RESULTS

The results are shown in Table I. The growth of BDC was inhibited when the cells were cultured with undiluted conditioned medium for both assays. Cell counts demonstrated a 27% decrease in cell numbers compared with the controls. The tritiated thymidine assay showed a 49% decrease in DNA synthesis compared with controls. This inhibition by the myeloma conditioned medium (MCM) was dose dependent (Fig. 1). Undiluted myeloma conditioned medium inhibited DNA synthesis in the BDC by 42% whereas the highest dilution (1/25) demonstrated no such inhibition. Fractions of MCM also inhibited DNA synthesis in the cells, with the <50,000 Da fraction showing more inhibitory activity than the >50,000 Da fraction (test/control = 0.064 compared with test/control = 0.121). The unfractioned MCM inhibited DNA synthesis less than either fraction (test/control = 0.173).

The inhibiting activity of the MCM was found to be dependent upon the density of the myeloma cells conditioning the medium. At least 1×10^6 cells per ml were necessary to produce inhibitory activity. No significant difference was seen in the growth of the BDC in the two different control media (*t*-test, $p < 0.5$, $n = 23$) and thereafter, RPMI only was used as a control.

Table I. Results of incubation with medium conditioned by myeloma cells, expressed as a ratio of test/control (see text)

Patient	Cell count n = 10	DNA synthesis n = 12
1	0.67	–
2	0.72	–
3	0.62	–
4	–	0.79
5	0.40	0.36
6	0.80	0.75
7	0.68	0.44
8	0.72	0.85
9	–	0.33
10	0.82	–
11	0.84	–
12	–	0.09
13	1.03	0.11
14	–	0.60
15	–	0.77
16	–	0.41
17	–	0.58
	$\bar{x} = 0.73 \pm$ s.d. 0.16*	$\bar{x} = 0.51 \pm$ s.d. 0.26†

* *t*-test, p 0.01
† Wilcoxon rank sum test, $p < 0.005$

Table II. Results of incubation with myeloma cells at $0.5 \times 10^4/cm^2$, expressed as a ratio of test/control (see text)

Patient	Cell count n = 10	DNA synthesis n = 12
1	0.61	–
2	0.69	–
3	0.62	–
4	0.68	–
5	0.89	1.09
6	0.68	–
7	0.86	–
8	0.86	–
9	–	1.01
10	–	1.10
11	–	1.17
12	–	1.14
13	–	1.61
14	0.70	0.89
15	0.93	0.68
16	–	0.96
17	–	1.02
18	–	0.89
19	–	1.11
	$\bar{x} = 0.75 \pm$ s.d. 0.12*	$\bar{x} = 1.06 \pm$ s.d. 0.22

* *t*-test, $p < 0.05$

Incubation of BDC with viable myeloma cells at $0.5 \times 10^4/cm^2$ showed a 25% inhibition of cell growth compared with controls (Table II), but no inhibition of DNA synthesis was seen. Similarly, no inhibition of DNA synthesis was seen in BDC incubated with myeloma cells at a range of cell densities from 0 to $5 \times 10^4/cm^2$, where test/control ratios were between 0.99 and 1.19.

DISCUSSION

Our results suggest that myeloma secretes a factor which inhibits osteoblast proliferation and that this could be responsible for the failure of the host bone to react to the myeloma-induced osteolysis. It has previously been shown that myeloma cells secrete osteoclast-activating factors (Mundy et al. 1974; Raisz et al. 1975) and it is reasonable to postulate that these cells may secrete other humoral factors as well. The effect is dose-dependent, substantiating our suggestion that the inhibition of the bone-derived cell proliferation is due to a soluble factor secreted by the myeloma cells. This is supported by the finding that a concentration of 1×10^6 myeloma cells in the conditioning medium was necessary for a positive inhibitory effect. Our results also suggest that the molecular weight of this factor is less than 50,000 Da.

The results help to explain why myeloma frequently gives false negative results on skeletal scintigraphy. Uptake of bone seeking radionuclides is dependent on the osteoblastic response to noxious stimuli. This tends not to occur with myeloma and, therefore, there is no increased uptake of isotope. It has also been shown that osteoclast-activating factor inhibits collagen synthesis in osteoblasts (Rossi and Bataille 1984). This suggests that myeloma may affect osteoblast metabolism as well as proliferation.

This project is supported by the Cancer Research Campaign.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES

Ashton BA, Abdullah F, Cave J, Williamson M, Sykes BC, Couch M, Poser JW. Characterization of cells with high alkaline phosphatase activity derived from human bone and bone marrow: preliminary assessment of their osteogenicity. *Bone* 1985;6:313–9.

Evans CE, Galasko CSB, Ward C. In vitro proliferation of cells cultured from human cancellous bone. In: Ali SY, ed. *Cell mediated calcification and matrix vesicles.* Amsterdam: Elsevier Science Publishers BV, 1986:303–7.

Galasko CSB. *Skeletal metastases.* London: Butterworths, 1986.

Gallagher JA, Beresford JN, Sharrard M, et al. Human bone cells in culture: a novel system for the investigation of osteoblast function. *Calcif Tissue Int* 1983;35 [Suppl]:A24.

Mundy GR, Raisz LG, Cooper RA, Schechter GP, Salmon SE. Evidence for the secretion of an osteoclast stimulating factor in myeloma. *N Engl J Med* 1974;291:1041–6.

Raisz LG, Luben RA, Mundy GR, et al. Effect of osteoclast activating factor from human leukocytes on bone metabolism. *J Clin Invest* 1975;56:408–13.

Rossi J-F, Bataille R. *In vitro* osteolytic activity of human myeloma plasma cells and the clinical evaluation of myeloma osteoclastic bone lesions. *Br J Cancer* 1984;50:119–21.