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Original Paper

Myeloma Bone Disease

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INTRODUCTION

MYELOMA BONE disease was first described in 1848 in a London tradesman called Thomas Alexander McBean [1]. The patient was a 42 year old man who suffered a severe and debilitating form of bone disease which was called by physicians *mollities and fragilitas ossium* (disease with soft bones). He was found to have a newly identified substance in his urine which had special chemical properties and which subsequently came to be known as Bence Jones protein [2]. It is now recognised to be a consequence of the presence of monoclonal free light chains of immunoglobulin molecules produced by myeloma cells.

This interesting and unique form of bone disease was first called multiple myeloma by Rusitzky [3] and later in Europe became and still is widely referred to as 'Kahler's Disease' [4]. It is now recognised as a neoplastic disease of plasma cells, with the neoplastic clone representing cells at different stages in the plasma cell lineage from patient to patient. The disease is characterised in many patients by impaired humoral immunity, susceptibility to infection, bone marrow effects due to the presence of marrow neoplastic cells and renal failure. However, the most striking feature in the majority of patients from the time of presentation is a unique form of crippling bone disease which was so evident in the first patient.

Myeloma has been a very informative disease for biomedical investigators. For example, our knowledge of humoral immunity and immunoglobulin production has been facilitated by observations made in patients with myeloma. Hybridoma technology for monoclonal antibody (MAb) production was first made possible with the use of murine myeloma cells and now the gross distortions of bone remodelling in human myeloma are providing insights into normal bone remodelling.

EPIDEMIOLOGY OF MYELOMA BONE DISEASE

The annual incidence rate for myeloma is approximately 5–10 per 100 000 population. The disease occurs mostly in the elderly and patients under the age of 40 years are uncommon. It is more prominent in Blacks than in caucasians (ratio 2:1) and slightly more common in males than in females.

There is a probable real increase in incidence of the disease in recent years. There are no aetiological factors that are well-identified. It is probably grossly under-registered, which makes assessment for risk factors difficult. However, there is no apparent clustering and no clear-cut familial incidence. Since the average life-span for patients with myeloma is approximately 3 years and over 90% of patients have overt bone disease, the approximate prevalence of myeloma bone disease in the United States at any point in time is approximately 30 000 cases. Median survival is 3 years, although there is great variability in life-span following diagnosis. Approximately 15–20% of patients survive 5 years, 7% 10 years and 1–2% of patients are alive at 15 years. No convincing cures have as yet been reported.

NATURE OF THE BONE DISEASE

Bone remodelling is profoundly disturbed in almost all patients with myeloma. Although occasional patients with other malignancies have skeletal manifestations, they are rarely as severe and certainly not as common as in myeloma. Bone destruction in myeloma is responsible for the most prominent and distressing clinical features of this disease. Eighty per cent of patients present with bone pain as a predominant symptom [5]. Patients with myeloma bone disease are susceptible to fractures occurring either spontaneously or following trivial injury. The bone pain is often intractable, but occasionally fluctuates in intensity for reasons which are unknown. Pathological fractures may involve the vertebrae, ribs and long bones (most commonly), but occasionally occur in other sites such as the sternum and pelvis. Hypercalcaemia occurs in approximately 30% of patients and is accompanied by its attendant symptoms and signs and usually by concomitant renal failure. The bone lesions occur in several patterns. Occasionally, patients develop single osteolytic lesions that are associated with solitary plasmacytomas. Some patients have diffuse osteopenia, which mimics the appearance of osteoporosis (albeit with some important radiological differences) and is due to the myeloma cells being spread diffusely throughout the axial skeleton. In most patients, there are multiple discrete lytic lesions occurring at the site of deposits or nests of myeloma cells.

Hypercalcaemia occurs as a consequence of bone destruction in approximately one-third of patients with advanced disease. Rarely, patients with myeloma do not have lytic lesions or bone loss, but rather have an increase in the formation of new bone around myeloma cells. This rare situation is known as osteosclerotic myeloma.

PATHOPHYSIOLOGY OF BONE LESIONS

Osteolytic bone lesions are by far the most common bone lesions in patients with myeloma. Although the precise molecular mechanisms remain unclear, observations over the past 30 years have revealed that:

- Bone is destroyed in myeloma by osteoclasts, the normal bone resorbing cell. This is the only cellular mechanism for bone destruction which is clearly evident in myeloma.
- Osteoclasts accumulate on bone resorbing surfaces in myeloma adjacent to collections of myeloma cells. Thus, it appears that the mechanism by which osteoclasts are stimulated in myeloma is a local one, whereby myeloma cells (or host cells) produce local factors (cytokines) responsible for increasing osteoclast formation and activation.
- It has been known now for many years that cultures of human myeloma cells *in vitro* express and secrete several osteoclast activating factors into the cell culture media. Several of these local mediators have been identified and include lymphotoxin (tumour necrosis factor β), interleukin-1β and interleukin-6. The mediator secreted appears to depend on the culture conditions.
- Hypercalcaemia occurs in approximately one-third of patients with myeloma sometime during the course of the disease. Hypercalcaemia is always associated with markedly increased bone resorption and frequently with impaired renal function which is fixed and due to the effects of the disease on renal function. Glomerular filtration may be further compromised by volume depletion and hypercalcaemia.
- Bone formation rates are often reduced in myeloma and the increase in osteoclastic bone resorption in myeloma is usually associated with impaired osteoblast function. Alkaline phosphatase activity in the serum is decreased or in the normal range, unlike patients with other types of osteolytic bone disease, and radionuclide scans do not show evidence of increased uptake, indicating lack of an osteoblast response to the increase in bone resorption, in contrast to what is found in other types of osteolytic bone disease such as breast cancer.
- Occasional patients with myeloma show predominantly an increase in new bone formation with subsequent osteosclerosis. This is often associated with the POEM's Syndrome (see below).

Bartl and associates [6] and Bartl and Frisch [7] have classified the morphological pattern of myeloma in the bone marrow and correlated the patterns they observed with the presence of osteolysis and survival. The patterns they identified are: (1) interstitial infiltration of the marrow; (2) interstitial with sheets of cells; (3) interstitial infiltration of the marrow with myeloma cells but with additional discrete nodules; (4) a nodular pattern alone; and finally (5) diffusely packed bone marrow. Interstitial and interstitial with sheet patterns are associated with the lowest frequency of osteolysis and nodular packed with the highest. The latter are also associated with the poorest prognosis. Not all morphologists agree that these patterns really represent different types of myeloma behaviour and it is not clear what the usefulness of this sort of classification is at the present time. However, it does emphasise again that there may be important interactions which occur between myeloma cells and neighbouring cells in the marrow micro-environment.

POTENTIAL FACTORS INVOLVED IN BONE DESTRUCTION IN MYELOMA

The pathogenesis of bone lesions is related to increased production of local cytokines in the involved bone marrow of patients with myeloma. This bone resorbing activity has similar characteristics to the bone resorbing activity produced by activated peripheral blood leucocytes that was formerly called osteoclast activating factor or OAF. OAF represents a family of bone resorbing factors that are produced by normal lymphocytes and monocytes following exposure to an antigen to which they have previously been exposed or a nonspecific mitogen such as phytohemagglutinin [8]. The OAFs which have been implicated in myeloma are lymphotoxin, interleukin-1β and interleukin-6.

Lymphotoxin

Lymphotoxin is a normal activated lymphocyte product which is also produced by lymphoid cell lines in culture and in particular by B-lymphoblastoid cell lines. It has now been found that in a number of cell culture lines isolated from patients with myeloma, the tumour cells express lymphotoxin messenger RNA and contain biological activity in the conditioned media which can be ascribed to lymphotoxin [9]. The conditioned media also contains bone resorbing activity which can be partially neutralised by lymphotoxin neutralising antibodies.

Lymphotoxin increases bone resorption [10] and stimulates the formation of osteoclasts from precursors in marrow cell cultures [11]. Moreover, lymphotoxin activates mature isolated osteoclasts to form resorption pits on bone slices [12]. Lymphotoxin has identical effects to those of tumour necrosis factor, interleukin-1α and β on bone resorption. Repeated injections of recombinant human lymphotoxin cause hypercalcaemia in normal mice [9].

Interleukin-1β

Interleukin-1α and β are powerful stimulators of osteoclastic bone resorption [13–15] and in addition cause hypercalcaemia *in vivo* through this mechanism [16, 17]. Freshly isolated marrow cells from patients with myeloma, which contain both myeloma cells and stromal cells, have been shown to produce interleukin-1β in the conditioned media [18, 19]. Bone resorbing activity produced by these cultures can be neutralised by antibodies to interleukin-1β. In contrast, established cell lines from patients with myeloma do not express interleukin-1β [9, 20]. The reason for these discrepancies probably relates to the nature of the cells which are studied. Artefacts could occur in both models. Established cell lines could have changed in culture to produce factors that the parent cells *in situ* did not. Alternatively, the freshly isolated cells (which contain dead and dying elements) probably release factors that they are not released *in situ* (as has been shown previously for prostaglandins).

Interleukin-1 stimulates the formation of osteoclasts from progenitor cells [11]. Interleukin-1 also activates mature isolated osteoclasts to resorb bone *in vitro* [21].

Interleukin-6

Interleukin-6 is a multifunctional cytokine which may play an important role in the pathophysiology of myeloma. There

is considerable evidence that suggests it may be an important growth factor in myeloma, and neutralising antibodies to interleukin-6 may have important effects on the course of the disease [22–24]. We have found that interleukin-6 has effects on bone resorption and calcium homeostasis which are different from those of interleukin-1 and tumour necrosis factor *in vitro* and *in vivo* [25]. Interleukin-6 does not stimulate osteoclastic bone resorption in organ cultures of fetal rat long bones or neonatal mouse calvariae. However, in other types of organ culture it has been shown to stimulate osteoclastic bone resorption [26, 27]. Interleukin-6 causes mild hypercalcaemia *in vivo*. When interleukin-6 is stably transfected into Chinese hamster ovarian cells, these cells from tumours in nude mice express interleukin-6. Mice carrying tumours with CHO cells expressing IL-6 develop increasing levels of IL-6 in the serum as the tumour grows. These mice become progressively hypercalcaemic and in addition develop leucocytosis, thrombocytosis and cachexia [25].

Interleukin-6 may also have effects in the bone microenvironment which are different from those of the other cytokines. Although bone cells isolated from trabecular bone surfaces (bone lining cells) express cytokines such as interleukin-1, tumour necrosis factor, colony stimulating factors and interleukin-6, it is only in the case of interleukin-6 that these bone cells produce more of a cytokine when exposed to osteotropic factors such as parathyroid hormone, interleukin-1 and tumour necrosis factor [28]. In the case of the other cytokines, production by bone cells may be enhanced by non-physiological stimuli such as lipopolysaccharide. In addition, bone cell expression of interleukin-6 can be decreased by incubation of the bone cells with oestrogen [29, 30].

The most important of these cytokines in bone lesions associated with myeloma remains unknown. It is possible that a combination of these cytokines work in concert to enhance bone resorption in myeloma, or that other factors are also involved. For example, in some patients, PTHrP has been implicated [31–33].

RELATIONSHIP BETWEEN BONE AND BONE CELL PRODUCTS AND MYELOMA CELLS

The observation that myeloma cells cause bone destruction by producing factors which stimulate osteoclasts to resorb bone has led to attention being focused on the identification of the responsible mediators. However, interactions between myeloma cells and bone cells are probably more complicated than simple excess production of a factor by myeloma cells which stimulates osteoclasts. Recent data have suggested that the avidity with which myeloma cells grow in bone compared with other haematological malignancies may be influenced by products produced as a consequence of osteoclastic bone resorption and in particular the cytokine interleukin-6, which is a major growth regulatory factor for myeloma cells. Thus, bone may not be simply a passive bystander in this disease, but rather may act to amplify the growth of myeloma cells in bone. If this concept is correct, then a vicious cycle could exist between myeloma cells and osteoclastic bone resorption, whereby myeloma cells stimulate osteoclasts to resorb bone by the production of osteotropic cytokines, such as tumour necrosis factor β, interleukin-1β and interleukin-6 (IL-6), but that, as a consequence of this increase in osteoclast activity, IL-6 is generated in large amounts by cells involved in the resorption process and this enhances the growth of myeloma cells in bone. This vicious cycle could mean that the greater the bone destruction, the more aggressive the behaviour of myeloma cells, which then may cause even greater bone destruction.

This concept is based on recent information that osteoclasts produce considerably more IL-6 than any other cell and certainly more than other types of bone cells. The only cells which produce comparable amounts of IL-6 are endometrial cells. However, osteoclasts are much fewer in number than are other cells. It is possible that production of IL-6 in the bone micro-environment may be related to direct cellular interactions between myeloma cells and other cells such as stromal cells, osteoblasts or even osteoclasts [34].

ANIMAL MODELS OF HUMAN MYELOMA BONE DISEASE

One major drawback to studying the mechanisms responsible for myeloma bone disease has been a lack of suitable animal models of the human disease. In contrast to solid tumours associated with hypercalcaemia of malignancy, human myeloma cells do not grow well in nude mice and it has not been easy to mimic the human disease in animal models. This has meant that it has been difficult to establish an acceptable animal model of the human disease to study pathogenetic mechanisms and determine the efficacy of various forms of therapy. However, the recent development of two animal models of human myeloma bone disease in recent years may circumvent these problems. One of these models is the murine model of myeloma described by Radl and associates [35]. Radl and coworkers have described a murine model of myeloma which occurs spontaneously in ageing mice of the C57 BL/KaLwRij strain. Myeloma occurs in these mice at the rate of approximately 1 in every 200 as they age and causes a monoclonal gammopathy with features reminiscent of the human disease, including bone marrow myeloma cells and most important for this discussion, characteristic myelomatous skeletal lesions. Osteolytic lesions are found in most mice and, in occasional mice, osteosclerotic lesions are found. We [24], as well as Manning and Colleagues [36], have developed cell lines from these myeloma cells which can be studied both *in vitro* and *in vivo*. Either freshly dispersed myeloma cells from the bone marrow or involved organs of myeloma-bearing mice can be transplanted into fresh mice by tail vein injection into the recipients of the same strain, or by bone marrow inoculation. The disease is faithfully transmitted from mouse to mouse. In order to develop a more convenient animal model of the human myeloma bone disease, the cell line that we have established from tumour-bearing mice was subcloned and fully characterised [24]. The cell line causes characteristic osteolytic bone lesions in mice when injected via the tail vein. The cell line also produces the monoclonal protein and IL-6, but its growth is independent of exogenously added IL-6. Some mice carrying these myeloma cells become mildly hypercalcaemic, again reminiscent of the human myeloma bone disease. The osteolytic bone lesions are characterised by an increase in osteoclast numbers and activity. Identical results are found when these tumour cells are inoculated into SCID (NU/BG/XID) mice. We have also been involved recently in the development of a second animal model of human myeloma bone disease [20]. In this model, human myeloma cells are inoculated into immunocompromised mice. These mice have severe combined immunodeficiency (SCID) and are irradiated. Under these circumstances, ARH-77 human myeloma cells cause typical osteolytic bone

lesions characteristic of myeloma in addition to mild hypercalcaemia. Mice carrying ARH-77 cells also develop hind limb paralysis 28–35 days after tumour cell inoculation.

HYPERCALCAEMIA IN MYELOMA BONE DISEASE

Hypercalcaemia in myeloma is due primarily to increased osteoclastic bone resorption caused by local cytokines released by the myeloma cells, which in turn leads to osteolysis and entry of calcium into the extracellular fluid. This entry of the calcium into the extracellular fluid overwhelms the patient's capacity to maintain normal calcium homeostasis and the result is hypercalcaemia. However, the pathogenesis of hypercalcaemia is even more complex than this. Firstly, not all patients with myeloma bone disease develop hypercalcaemia [5]. Approximately 20–40% of patients develop hypercalcaemia, usually late in the course of the disease, but not always. This frequency may be decreasing slightly in recent years with the advent of bisphosphonate therapy. By-and-large, hypercalcaemia occurs in those patients who have the largest tumour volume, although not all patients with large tumour burdens develop hypercalcaemia. The reasons for this are unclear, but may be related to the amount of bone resorbing activity produced by the myeloma cells, as well as the status of glomerular filtration. We have found that measurements of total body myeloma cell number together with production of bone resorbing activity by cultured bone marrow myeloma cells *in vitro* do not correlate closely with hypercalcaemia, although they do correlate with the extent of osteolytic bone lesions [37]. Thus, there are clearly other factors which are involved in the pathogenesis of hypercalcaemia in addition to osteoclast activation. Probably the most important of these is the impairment of renal function which occurs frequently in patients with myeloma. Impaired renal function in myeloma occurs for multiple reasons, including uric acid nephropathy, amyloid nephropathy, myeloma kidney due to Bence Jones protein excretion, chronic pyelonephritis and hypercalcaemia itself. In addition to impaired glomerular filtration, increased renal tubular calcium re-absorption may also be a contributing factor to the pathophysiology of hypercalcaemia [38]. It is unclear why patients with myeloma have this increase in renal tubular calcium re-absorption. The differences between hypercalcaemia which occurs in myeloma and hypercalcaemia which occurs in patients with solid tumours is sometimes of assistance in the differential diagnosis. For example, in patients with hypercalcaemia due to myeloma, there is almost always impaired renal function and an increase in the serum phosphorus which is associated with decreases in glomerular filtration rate. Markers of bone formation such as serum alkaline phosphatase are usually not increased in patients with myeloma, since bone formation is not increased and in fact may be impaired for reasons which are not clear. Patients with hypercalcaemia due to myeloma usually respond very rapidly to treatment with corticosteroids and calcitonin, unlike patients with hypercalcaemia due to solid tumours [39].

BONE MARKERS IN MYELOMA BONE DISEASE

Bone markers may eventually be important for monitoring the progress of osteolytic bone disease. The current most useful marker for bone resorption is the measurement of deoxypyridinoline crosslinks of collagen [40]. These can be readily measured in the urine either by chemical assay or by ELISA. Hopefully this marker may eventually be measurable in the serum. This measurement provides a very accurate and precise parameter of osteoclastic bone resorption which is much improved over previous markers such as urinary hydroxyproline or fasting urine calcium.

Parameters of bone formation such as serum alkaline phosphatase are often decreased in patients with myeloma, unless the patient has an active fracture undergoing repair. Measurements of osteocalcin (bone GLA protein) show a large scatter. Serum osteocalcin is usually decreased in patients with advanced disease and more extensive bone lesions. Earlier in the disease in patients who have less aggressive or obvious bone disease, serum osteocalcin levels may be normal or even increased [41].

TREATMENT OF MYELOMA BONE DISEASE

For a number of years, clinicians have attempted to devise therapeutic approaches in myeloma that would relieve disabling symptoms due to skeletal destruction. This was first attempted with the use of fluoride and later calcium and fluoride, although this combination was ineffective and, in fact, probably detrimental because of the associated side-effects. More recently, several groups have shown that the newer generation bisphosphonates will relieve bone pain and produce a rapid, sustained and significant decrease in the urinary excretion of calcium and hydroxyproline indicating decreased bone turnover [42, 43]. This was shown first with pamidronate [42] and then with clodronate [43]. Pamidronate has recently been approved by the FDA for this use in myeloma patients without hypercalcaemia. A large study by Berenson and associates [44] showed that in several hundred patients with myeloma, there was a satisfactory response in bone pain, reduced need for radiation therapy, and prevention of fractures. This study confirmed other large studies in Europe and the results of the sixth MRC myeloma trial, in which clodronate was the bisphosphonate used (E. McCloskey, University of Sheffield, U.K.). Outstanding questions include which is the best bisphosphonate for this purpose, what is the ideal dose, for how long should it be administered, should it be given to patients early in the course of the disease and most importantly do these drugs have a beneficial effect on survival. Active research in this area is likely soon to lead to the introduction of other suitable and nontoxic agents of this class that will be useful as oral forms of therapy to relieve the symptoms caused by bone destruction and its complications in patients with myeloma.

There are other important issues in the management of myeloma bone disease. This crippling form of bone disease is associated with the most severe and intractable bone pain, as well as frequent fractures following trivial injury. When the bone pain is localised to specific areas such as in the vertebral spine or the ribs, a course of local radiation therapy may be very effective. Analgesics should be used liberally for severe pain. Patients require frequent counselling because of the bone pain, deformity and loss of height associated with progressive myeloma bone disease and will in general manage their symptoms best when they understand the nature of the bone disease and those activities which put them at risk for further complications. Similar principles that guide the lifestyles of patients with osteoporosis also apply to myeloma bone disease. For example, patients should avoid those

situations that are risky for the development of fractures such as climbing ladders, slipping on ice or slipping on loose bathroom rugs. They should also be made aware of local support groups which are located in some of the world's major cities, as well as the International Myeloma Foundation, which was founded in 1990 and is dedicated to improving the quality of life for multiple myeloma patients. This Foundation has a 'hot line' and runs seminars for patients and family members as well as physicians and health professionals and serves as a resource centre for information for patients with this unfortunate condition.

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