

The Cellular Basis for Extremity Bone Loss in Leprosy^{1,2}

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Our understanding of the pathogenesis of the skeletal effects of leprosy has lagged behind our understanding of the neurologic (33, 34) and immunologic (35, 36) events in the disease. This has not been due to lack of effort but to the difficulties inherent in any study of the skeletal system; namely, difficult access and delayed development of methods to study cell structure and function in mineralized tissues (27).

Skeletal involvement is a serious and probably universal feature of leprosy (24, 28, 29, 31, 32, 41, 42) and results in a net loss of skeletal mass. Bone changes are described as specific, in which acid-fast bacilli are demonstrable, or nonspecific in which mycobacteria can be found. Specific bone lesions are relatively rare, occur in the lepromatous forms and are confined to the small bones of hands, feet and nose. The overwhelming majority of skeletal lesions are nonspecific and are of four general types: distal absorption of the digits, osteoarthritis, osteomyelitis and osteoporosis (24, 32).

Because a frequent major disability of leprosy is anesthesia and paralysis, skeletal changes have been considered to be of neurovascular origin (4, 15, 31). The effects of the mycobacteria on peripheral nerves were believed to result in loss of vascular tone, hyperemia and bone resorption. While this sequence of events may occur in some patients, it appears to be far from universal. Serial arteriograms in more than 60 patients have shown proximal arteriovenous shunts and reductions of blood flow in digital arteries (7, 32). In a study of new patients admitted to Carville, no bone changes could be directly attributed to sensory loss (10). Furthermore,

denervation of extremities in experimental animals has not produced bone resorption (13). The development of bone loss in leprosy is a complex process and probably involves some type of circulatory alteration on which is superimposed infection, inflammation, trauma or disuse (10, 32, 37).

The mechanism of skeletal loss in leprosy has been studied primarily by radiographic and histologic methods. Numerous radiographic studies have described the progressive loss of skeletal mass in the distal extremities and nasal septum (3, 6, 8, 10, 15, 24, 32, 41, 42). The most revealing information has come from combined radiographic and histologic studies (2, 21, 22, 32). However, there is still a basic uncertainty as to how the bone loss occurs. Aseptic bone necrosis is found in leprosy but not frequently enough to be a major mechanism (32). Sections of bone from patients with leprosy, on the other hand, show few bone cells and a fibrotic marrow. Osteoclasts, an accepted cellular source of bone resorption, are reported to be either absent or of unusually rare occurrence (2, 21, 22). A significant event in the search for a cellular basis for bone loss in leprosy was the demonstration by biochemical methods (11) that the concentration of the lysosomal enzyme acid phosphatase was significantly higher in bone from patients with resorptive bone lesions than in bone from patients without much bone loss.

Because the extracellular release of acid phosphatase by osteoclasts is known to be a cellular event in bone resorption (25, 27, 39), we have pursued the cellular basis of bone loss in leprosy by combining a method to study the cellular distribution of acid phosphatase activity with excellent preservation of cytologic detail in demineralized bone sections. This report demonstrates that osteoclasts are present in patients with lepromatous and tuberculoid leprosy and that they are indistinguishable morphologically from normal, active osteoclasts. These results suggest that bone loss in leprosy results from acceleration of a physiologic event and not another process such as aseptic bone necrosis.

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MATERIALS AND METHODS

Methods. Bone was obtained from patients undergoing lower limb amputation or other surgical procedure designed for the patient's benefit at the National Leprosy Control Center, Sungei Buloh, Malaysia. The type of leprosy was determined at the National Leprosy Control Center by smears and clinical examination (23). When possible bone was taken from each patient from areas of resorbing and nonresorbing bone as determined on preoperative X-rays, from areas with and without infection and from normal bone not involved in the disease.

Bone specimens were removed, cut into pieces of less than 2 mm in at least one dimension and immersed in chilled 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, within ten minutes of interrupting the blood supply. All specimens were fixed in this solution for two hours, washed briefly with buffer and demineralized in 10% EDTA in 0.1 M tris buffer at 4°C for four to seven days (12). Bone samples were then embedded in 5% agar at 39°C and cut at 100-200 μ (38). At this stage demineralized sections from some specimens were fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer and embedded in epoxy resin. One and two micron sections were stained with toluidine blue.

Acid phosphatase activity in sections from each area was demonstrated as follows. Demineralized sections were washed overnight in cacodylate buffer and suspended in 1% gum acacia in 0.22 M sucrose at 4°C for two to four days (16). Enzyme activity was localized by the Gomori (14) procedure as modified by Barka and Anderson (1) with β -glycerophosphate as substrate. After incubation at 37°C for 30 minutes, the sections were washed and reacted with dilute lead sulfide. Osmication and embedment of these sections in epoxy resin and staining were as described above. Specificity of the reaction was determined using the following controls: incubation without substrate, incubation in complete medium containing an enzyme inhibitor (0.1 M sodium fluoride) and the use of heat-inactivated sections (90°C for 30 minutes).

Patient material. *Patient A* was a 60 year old Indian male who had had lepromatous leprosy for more than 30 years. He suffered from recurrent plantar ulcers on both feet

and two years earlier the right forefoot and left hallux were amputated. He had a large, deep ulcer of the medial aspect of the sole of the remaining part of the foot. Preoperative X-rays revealed absence of metatarsals and resorption of the cuneiforms and cuboid. A deep erosion of bone at the level of the talonavicular joint indicated the site of the ulcer. The tibiotalar and subtalar joint spaces were irregularly narrowed. The calcaneus was radiolucent and its trabeculae poorly defined. Subperiosteal bone formation was evident along the posterior margin of the distal tibia. The tibia and fibula were otherwise unremarkable. The right leg was amputated below the knee and bone samples were taken from resorbing tarsal bones near and away from the plantar ulcer, the lateral part of the talus, proximal calcaneus, distal tibia and fibula and tibial shaft.

Patient B was a 77 year old Chinese male who had had lepromatous leprosy for over 30 years. He was currently under treatment but his medical history was one of follow-up failure. He had ulcers on both forearms and feet. There were five interconnecting sinuses around the metatarsophalangeal (M-P) joint of the right hallux. Preoperative radiographs of the right foot revealed extensive resorption of the distal and lateral end of the first metatarsal and adjacent part of the proximal phalanx. A central radiolucency was present at the junction of the distal and middle thirds of the first metatarsal. The distal phalanx of the right hallux showed subperiosteal resorption and erosion of the tuft. The right hallux was removed, the ulcer crater curetted and bone samples were taken from two areas of the first metatarsal, three areas of the proximal phalanx, and four areas of the distal phalanx.

Patient C was a 63 year old Chinese male who had been treated for lepromatous leprosy for more than 20 years. The second toe on his right foot was removed three years earlier because of extensive ulcerations. Preoperative X-rays of the right foot showed collapse of the distal tarsal row, thinning of the shaft of the proximal phalanx of the great toe, extensive resorption of the phalanges of the third toe, and resorption of the middle and distal phalanges of the fourth toe (Fig. 1). A below-knee amputation of the right leg was performed and 16 bone samples were taken from areas marked by as-



FIG. 1. Preoperative X-ray of distal part of right foot of patient C. The second toe was removed three years earlier because of extensive ulceration. Areas of bone sampled for cytochemical study are indicated by asterisks. There is extensive resorption of phalanges of the third toe.

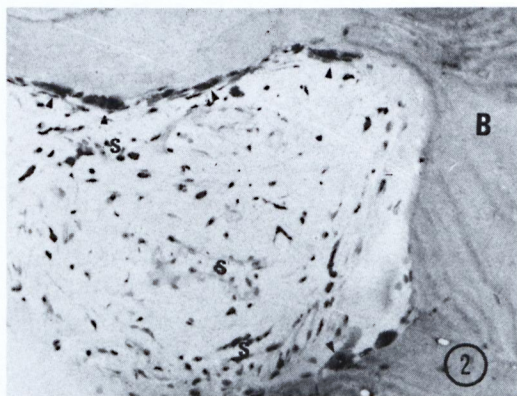


FIG. 2. Low power photomicrograph of seven osteoclasts (arrow heads) which show up in bold outline next to the bone (B) margin because of their high concentrations of acid phosphatase activity. Venous sinuses (S) are prominent features of the loose connective tissue. Proximal phalanx, third toe, patient C (counterstained with toluidine blue, $\times 160$).

terisks in Figure 1 and from the distal tibial and fibular metaphyses, talus and calcaneus.

Patient D was a 59 year old Indian female. She had had tuberculoid leprosy for 35 years and was released from treatment ten years ago. She had a 2×3 cm plantar ulcer on the right foot which had not responded to treatment. There was extensive resorption of the lateral part of the right foot and the fourth and fifth digits were absent. Preoperative radiographs of the right leg and foot revealed that the cuboid and lateral cuneiform were absent. There was generalized thinning of the cortices of the remaining metatarsals and collapsed M-P joints on the first two digits. Subperiosteal bone formation was present on the medial surface of the distal tibial metaphysis. A right leg amputation was performed below the knee and bone samples were taken from 16 sites on phalanges, metatarsals, tarsals and distal tibia.

Patient E was a 55 year old Malay male who had been treated for tuberculoid leprosy for 20 years. He had a chronic infection of the left tibio-talar joint. The left forefoot showed extensive resorption and had no digits. Preoperative X-rays showed that the proximal talus was resorbed and irregular, resulting in a greatly widened tibio-talar joint. Only the bases of the first three metatarsals remained and cuneiforms and cuboid were collapsed. The left leg was amputated 10 cm above the ankle and nine bone samples were taken from metatarsals, cuneiforms and distal tibia.

RESULTS

Microscopic study of the 60 bone specimens revealed osteoclasts in the majority of sections from each patient including areas of resorbing and non-resorbing bone as determined on preoperative radiographs. Many osteoclasts were present in small groups in isolated areas. However, large groups of osteoclasts were frequently seen over extensive areas of bone surface (Fig. 2). These cells could be identified even at relatively low magnification in the sections stained for acid phosphatase activity because of their high concentration of the lysosomal enzyme. The typical intracellular distribution of acid phosphatase in functioning osteoclasts is illustrated in Figure 3. The enzyme is concentrated in a linear array within lysosomes at the interface between cell and bone. Enzyme

activity is also seen in other parts of the cytoplasm, particularly in the circumnuclear Golgi apparatus where lysosomes originate. Often enzyme distribution in neighboring osteoclasts varied greatly (Fig. 4), perhaps representing age or functional differences between cells.

Osteoclasts were consistently seen in bone samples taken from metatarsals and phalanges, the sites of basic bone lesions of leprosy (^{2,32}), and in bones (tibial metaphysis and some tarsals) not involved in the disease. No morphologic or cytochemical differences were noted between osteoclasts in these locations. Osteoclasts were frequently absent in resorbed bone near ulcerations and infections and near fibrotic marrow spaces. These areas were composed histopathologically of large masses of lipid which were well preserved by the two fixatives used, by a chronic inflammatory cell infiltrate containing numerous mononuclear cells, many of which showed a strong reaction for acid phosphatase, and by fibrous connective tissue.

Osteocytes with enlarged lacunae were found near bone surfaces occupied by osteoclasts (Figs. 3, 4). These cells showed some reactivity for the enzyme acid phosphatase but the intensity of the reaction was much less than in adjacent osteoclasts.

Osteoclasts and osteocytes with enlarged lacunae were more frequently observed in the three patients with lepromatous leprosy than in the two patients with tuberculoid disease. Nevertheless, these cells were readily identified in the latter patients with the methods used.

DISCUSSION

It is known that bone resorption is produced by two groups of cells within the skeleton: osteoclasts and a subgroup of the osteocyte population, the osteolytic osteocytes (²⁷). Electron microscopy has shown that the osteoclast is the major cell responsible for bulk resorption of bone (⁵). Studies of osteocytes (^{18,19}) have revealed that this group of cells can be divided functionally into three subgroups: osteogenic osteocytes which function as osteoblasts; osteolytic osteocytes which function as osteoclasts; and resting osteocytes. Furthermore, modulation between any of these states by a single osteocyte is possible after an appropriate

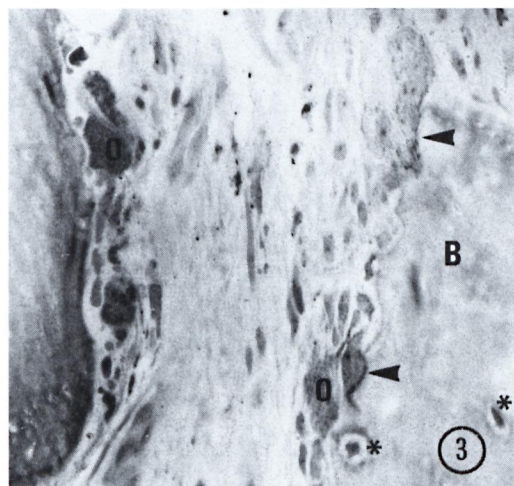


FIG. 3. Photomicrograph of osteoclasts (o) along adjacent resorbing surfaces (B) of phalangeal bone. Lacunae of adjacent osteocytes (*) are enlarged indicating resorption of lacunar walls. A concentration of acid phosphatase next to the bone surface is prominent in the two osteoclasts on the right (arrow heads). Distal phalanx, hallux, patient B (counterstained with toluidine blue, X 400).

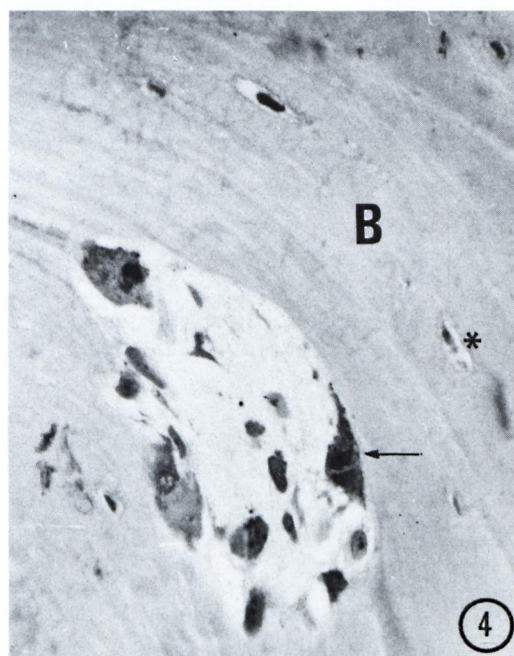


FIG. 4. Photomicrograph of three osteoclasts in a resorbing cone in compact bone. Acid phosphatase activity is greatest in one osteoclast (arrow). Vicinal osteocytes (*) exhibit lacunar enlargement. Tarsal bone away from site of infection, patient A (counterstained with toluidine blue, X 400).

change in its organelles (20). Osteolytic osteocytes and osteoclasts have the highest concentration of lysosomal enzymes (3, 25, 27) of the resident cells of bone.

The results of the present study demonstrate that osteoclasts and osteolytic osteocytes are present in areas of resorbing skeletal mass in patients with lepromatous and tuberculoid leprosy and that they have the enzymatic and structural features of functioning cells. These observations identify the cellular basis for previous reports of elevated levels of acid phosphatase activity in bone from patients with leprosy (11) and of micro-radiographic studies of bone resorption in adults with leprosy (9).

Osteocytes and osteoclasts were easily identified in sections stained cytochemically for acid phosphatase. The combination of acid phosphatase cytochemistry and the excellent preservation of cytoplasmic detail obtained by the fixatives and embedding agents used permitted identification of even small pieces of osteoclast cytoplasm because of its concentration of lysosomal enzymes. This method was a distinct advantage over the use of multinucleation, large cytoplasmic mass or presence of Howship's lacunae as identifying criteria for osteoclasts. This may account for our ability to find numerous osteoclasts when previous investigators (2, 21) failed to find many.

The observation by us and others of lymphoid and mononuclear cells near areas of bone resorption might be significant. Barnetson (2) noted that in older cases with continued bone loss there was increasing fibrosis of marrow. Job (21) described the development of bone lesions in the presence of leprosy bacilli. He found that large numbers of macrophages laden with ingested *M. leprae* and lymphocytes and plasma cells invaded and destroyed bone trabeculae and that healing of the defect was by fibrosis. We have observed mononuclear cells in areas of resorbing bone near infections. Recently investigators have reported bone resorption by mononuclear cells and acceleration of this process by a local factor released from stimulated lymphocytes (17, 30). This latter substance has been called OAF (Osteoclast Activating Factor) and has been shown to stimulate bone resorption in organ culture. Additional evidence of a role for lymphoid cells in bone resorption is the dem-

onstration that bone resorption can be restored and skeletal sclerosis cured in congenitally osteopetrotic mice by the transfer of spleen cells from a normal littermate (40). More recent evidence shows that one need only transfer lymphocytes, mononuclear cells and macrophages from normal spleen or thymus to effect a cure in a similar rat mutation (26). These latter observations suggest that lymphocytes and/or monocytes either transform directly into competent osteoclasts or that they elaborate some factor which allows them to function for the first time in these osteopetrotic mutants. Similarly, increased localized resorption of bone in some areas in patients with leprosy may be related to the presence of mononuclear cells, and the skeletal effects of the disease may be associated more closely with the known immune changes (35, 36) than we realize.

We suggest that bone resorption in leprosy is an acceleration of a normal process mediated via osteoclasts and osteolytic osteocytes and perhaps via monocytes. This acceleration of bone resorption might be caused by local release of some factors from *M. leprae* or from stimulated host cells. These hypotheses are approachable via culture of bone with *M. leprae* and/or cells from the host. The presence of acid-fast bacilli and their relationship to osteoclasts and osteolytic osteocytes in areas of bone damage should also be studied. The emerging relationships between bone resorption and the lymphoid system might be profitably explored in patients with leprosy.

SUMMARY

Osteoclasts and osteolytic osteocytes have been observed in the majority of 60 samples of bone taken from five patients with lepromatous or tuberculoid leprosy. These results are interpreted to mean that bone loss in patients with leprosy is an acceleration of a normal cellular process and not the result of avascular necrosis. The acceleration of bone resorption could be due to local release of products from *M. leprae* or host cells, a hypothesis testable by organ culture methods. The presence of lymphocytes and mononuclear cells in bone samples in this and previous studies is discussed with respect to recent evidence of a role for lymphoid cells in bone resorption.

RESUMEN

Se observaron osteoclastos y osteocitos osteolíticos en la mayoría de 60 muestras de hueso tomadas de 5 pacientes con lepra lepromatosa o tuberculoide. Estos resultados se han interpretado como indicación de que la pérdida del hueso en los pacientes con lepra es una aceleración del proceso celular normal y no el resultado de necrosis avascular. La aceleración en la reabsorción del hueso podría deberse a la liberación local de productos del *M. leprae* o de las células huésped, una hipótesis susceptible de probar por métodos de cultivo de órganos. Se discute la presencia de linfocitos y de células mononucleares en las muestras de hueso con respecto a las evidencias recientes del papel de las células linfoides en la reabsorción ósea.

RÉSUMÉ

On a prélevé 60 échantillons d'os chez 5 malades souffrant de lèpre lépromateuse ou de lèpre tuberculoïde; dans la majorité de ces échantillons, on a observé des ostéoplastes et des ostéocytes ostéolytiques. On interprète ces résultats comme un signe que la perte osseuse chez les malades de la lèpre constitue une accélération du processus cellulaire normal, et non point le résultat d'une nécrose avasculaire. L'accélération de la résorption osseuse pourrait être due à la libération locale de produits de *M. leprae* ou des cellules-hôtes, hypothèse qui pourrait être mise à l'épreuve par des méthodes de cultures d'organes. La présence de lymphocytes et de cellules mononucléaires dans les échantillons d'os, constatée dans cette étude, de même que dans les études antérieures, est discutée en rapport avec la constatation récente du rôle joué par les cellules lymphoïdes dans la résorption osseuse.

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