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Commentary

An infectious origin of extraskeletal calcification

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Apparently idiopathic extraskeletal calcifications are a common medical problem (Table 1). Approximately 7% of adult men develop renal or bladder stones containing calcium mineral salts (1). Life-threatening calcification may occur after hemodialysis, in scleroderma, and in patients with sclerotic aortic valves. The stimuli for the calcium salt deposition in these conditions are unclear, but nidi for precipitation and crystallization are needed even under supersaturation conditions.

In this issue of the *Proceedings*, Kajander and Çiftçioglu (2) show that a new class of bacteria, designated nanobacteria because of their small size (0.05–0.5 μm in diameter), produce sufficient calcium apatite to initiate pathologic calcification and stone formation. The nanobacteria were discovered in white films sticking to the surfaces of tissue culture vessels containing mammalian cells and media supplemented with bovine serum (3). A member of the Proteobacteria family, which includes *Bartonella* and *Brucella* species, the nanobacteria have distinctive properties, including heat resistance and the ability to pass through 0.1-μm sterilization filters (Table 2). Their most remarkable characteristic is the formation of carbonate apatite crystals at neutral pH and at physiologic phosphate and calcium concentrations. The extracellular mineralization forms a hard protective shelter for these hardy microorganisms, and it enables them to survive conditions of physical stress that would be lethal to most other bacterial species. Although it is not clear exactly how the nanobacteria induce calcification, other bacteria in aqueous sediments have been demonstrated to release oligopeptides that nucleate calcium apatite (4).

Proteobacterial infections are common in cows, and fetal bovine serum is the presumed origin of the tissue culture contaminants. Kajander and Çiftçioglu (5) have found that more than 80% of fetal bovine serum batches, each pooled from several thousand animals, have nanobacteria, as determined by immunoassay with monoclonal antibodies and by direct culture. Because nanobacteria are relatively resistant to the antibiotics commonly added to tissue culture media, it seems likely that many established cell lines might have a superimposed nanobacterial contaminant. Just as problems with mycoplasma and simian virus 40 infection have confounded tissue culture experiments in the past, so nanobacterial infestation could perversely influence the immunologic, metabolic, and growth properties of normal and malignant cells propagated *in vitro*. Such effects have already been reported, and the necessary technology to detect nanobacteria in tissue culture is emerging (6).

Recently, microbiologists have come to realize that blood can harbor close relatives of nanobacteria, without obvious pathologic sequelae for the natural host. For example, *Bartonella henselae* can be detected in the blood of almost half of the cats in the United States (7). Prior to pasteurization, human infections with proteobacteria, such as *Brucella melitensis* (formerly *Brucella abortus* and five other species), were much more common. It is still not known whether nanobacteria are present in cow's milk, whether the organism can survive current methods of sterilization, and whether human infection can be initiated by the oral route. However, nanobacterial infections do occur in people. An analysis of 30 demineralized kidney or bladder stones with two different monoclonal antibodies revealed nanobacterial antigens in every specimen (2). In some instances, sterile filtered extracts of the stones also grew pure nanobacteria cultures, which when injected intravenously into rabbits, localized preferentially to the kidneys (8). Renal stones are generally not considered to have an infectious etiology. However, the carbonate apatite released by inconspicuous nanobacteria colonies could certainly supply a nidus for calcium oxalate crystal formation.

Table 1. Some diseases associated with prominent idiopathic extraskeletal calification

Kidney and bladder stones
Dental pulp stones
Some gall stones
Salivary gland stones
Chronic calculous prostatitis
Testicular microliths
Calcification in hemodialysis patients
Atherosclerosis
Malacoplakia
Scleroderma (systemic sclerosis)
Calcinosis cutis
Calcific aortic stenosis
Several malignancies
Some dementias
Calcific tenditis and arthritis
Diffuse interstitial skeletal hyperostosis

Nanobacterial cultures have been established from human blood and urine. Patients on hemodialysis frequently receive multiple blood transfusions, and they may develop extensive extraskeletal calcifications. In unpublished studies, Kajander and Çiftçioglu (personal communication) have found that 80% of hemodialysis patients in a hospital in Turkey were nanobacteremic. It will thus be important to determine the exact frequency of nanobacterial infection of human blood, and whether the routine screening of blood donors is necessary.

Because they are hidden in mineral shelters, nanobacteria are difficult to eradicate with short-term antibiotic treatment. However, the tetracyclines have a known ability to accumulate on apatite, and at least are bacteriostatic to nanobacteria at clinically achievable concentrations. This property should tend to localize the antibiotic to nanobacteria, which always have apatite as part of the cell wall. Recent anecdotal reports claim a benefit for long-term tetracycline therapy in some patients with scleroderma, one of the most ominous diseases associated with extraskeletal calcification.

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Table 2. Unusual properties of the nanobacteria

Property	Description
Size	0.05–0.5 μm
Filterability	0.1 μm in high yield
Mineral formation	Biogenic apatite formation
Doubling time	≈3 days
Heat resistance	90°C, 1 h
Culturability	Mammalian cell culture medium with/without cells
γ-irradiation resistance	1.5 megarads
Antibiotic sensitivity	Resistant to penicillins and aminoglycosides
Diagnosis	Standard sterility detection methods fail

Data are from Kajander *et al*. (3).

Now that specific monoclonal antibodies and potential nucleic acid probes are available, careful epidemiological studies should be able to establish the prevalence and consequences of nanobacterial infections in humans. Even if nanobacteria are not a primary cause of extraskeletal calcification in the various diseases listed in Table 1, they are a potentially treatable exacerbation factor. A role for bacterial infection in the pathogenesis of peptic ulcer disease was established only recently, after years of inconclusive research. Tantalizing recent data also suggest a role for bacterial or viral infection in the host inflammatory response to atherosclerotic vascular damage (9). On the basis of the early results of Kajander and Çiftçioglu, there is ample cause to investigate thoroughly the part that nanobacteria play not only in renal stone formation but also in the many perplexing diseases associated with pathologic extraskeletal calcification.

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