UNDER THE MICROSCOPE

Nanobacteria – propagating calcifying nanoparticles

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Abstract

Nanobacteria, also known as calcifying nanoparticles (CNP), are controversial infectious agents not matching the current criteria for 'living organism'. Despite the controversy of their classification, they propagate and cause cell death *in vitro* and are associated or found in many human diseases. Thus, more efforts should be focussed on research on pathogenicity of CNP.

Introduction

Nanobacteria appear as self-propagating calcifying macromolecular complexes found in bovine and human blood and blood products. These nanoparticles are active nidi (meaning that biomineralization is taking place out of chemical equilibrium), forming calcium phosphate mineral under subsaturation level of calcium and/or phosphate. Nanobacteria were found to be present in fetal bovine serum and cultured cell lines, to arouse immune response and to infect humans, and were published as an infectious cause for pathological calcification (Kajander and Çiftçioglu 1998). Several important human diseases have calcium phosphate deposition as a hallmark, e.g. atherosclerosis and cardiovascular diseases, stone formation in kidneys, gall-bladder, salivary, venous and gingival locations, other urological diseases, e.g. prostatitis, many cancers and various forms of autoimmune diseases and arthritis. A controversy exists in understanding the nature and thus the classification of nanobacteria. As the bacterial status of nanobacteria is still lacking satisfactory 16S rRNA sequence, the name calcifying nanoparticle (CNP) best describes the agent. Despite the controversy, or maybe inspired by controversy, research on CNP has been carried out up to the new millennium (Table 1).

The Pathogenicity

The disease-causing mechanisms of nanobacteria include known effects of calcium on blood vessels, blood coagulation and thrombus formation; elevation of intracellular $[Ca^{2+}]$ levels and its consequences (stimulation to apoptotic cell death or to uncontrolled growth potentially contributing to tumoural growth or malignancies); induction of autoimmune diseases, inflammation, arthritis and pathological calcification. CNP or blood calcium phosphate macromolecular complexes similar to CNP have been found so far in animals and humans by many researchers (see Table 1), and have been proposed as a treatable cause for atherosclerosis. It is notable that Cisar *et al.* (2000) recognized the presence of the macromolecular calcium phosphate complexes in blood, but regarded them as normal constituents of blood. Aoki and Aoki (1996) have shown that calcium phosphate (apatite) particles injected into blood circulation killed Wistar rats by acute thrombosis of blood vessels in lungs and other main organs. It is well known that exposed calcium phosphate in unstable atherosclerotic plaques can trigger thrombosis leading to myocardial infarction. Elevation of intracellular $[Ca^{2+}]$ promotes cell growth by bypassing growth-factor stimuli. Hence, calcium phosphate blood macromolecular complexes can be detrimental to human health. Self-replicating and infectious calcifying particles should be of great concern.

CNP interactions with bacteria

Co-culture of gram-negative bacteria with CNP showed rapid mixed-biofilm formation as exhibited by *Agrobacterium tumefaciens*, and adherence and internalization to *Escherichia coli* DH5 alpha (Fig. 1; Kajander *et al.* 2005). These results open new insights into CNP–bacteria

Table 1 Studies showing accumulating evidence on association of nanobacteria with human diseases

Year	
2000	Polycystic kidney disease cysts contain calcifying nanoparticles, (CNP) and this may contribute to cyst formation (Hjelle *et al.* 2000). Cisar *et al.* (2000) indicated US Food and Drug Administration (FDA) and National Institutes of Health (NIH) research interest, focussing on dead or alive issue, not for risk assessment. Garcia-Cuerpo *et al.* (2000) proved Koch's postulates for kidney stones.
2001	CNP contamination of vaccines reported at American Society for Microbiology Annual Meeting (Kajander *et al.* 2001). Centers for Disease Control and Prevention (CDC) and FDA acknowledged the presence of CNP in human and animal vaccines (Anon 2001). International Nanobacteria Minisymposium, Kuopio, Finland (http://www.nanobac.fi/nbminisymp080301/NB-minisymposium.html).
2002	Presence of CNP markers in commercial human gammaglobulin preparations reported at ASM Annual Meeting (Aho *et al.* 2002). CNP detected in human bile (Wen *et al.* 2002).
2003	Report on the presence of CNP in ovarian cancer with psammoma calcifications (Sedivy and Battistutti 2003). Detection of CNP in gall bladder tissue of gall stone patients (Wen *et al.* 2003). Nature of CNP addressed by Aho and Kajander (2003).
2004	Mayo Clinic published the presence of CNP in heart calcifications (Miller *et al.* 2004). Maniscalco and Taylor (2004) published first human trial on treating patients with coronary artery disease with anti-CNP treatment. CNP-caused mitral valve calciphylaxis was reported in human (Jelic *et al.* 2004). CNP found in Indian kidney stones (Khullar *et al.* 2004). Zhu *et al.* (2004) reported epidemiological studies indicating CNP as coronary artery calcification risk indicator. Presence of CNP in ovarian cancer calcifications proven by another Austrian group, 16S rRNA sequence supporting the first bacterial classification (Kajander and Çiftçioglu 1998) was repeated (Hudelist *et al.* 2004). CNP are present in HIV-infected mothers and babies (Pretorius *et al.* 2004). National Aeronautics and Space Administration (NASA) established Nanobacteria Laboratory.
2005	Therapy against CNP proven efficacy with chronic refractile prostatitis patients with unknown reason for disease (Shoskes *et al.* 2005). A study made at NASA showed that CNP growth is affected by gravity changes (Çiftçioglu *et al.* 2005). CNP contribute to bacterial biofilms (Kajander *et al.* 2005). CNP-like agents were cultured from human atherosclerotic plaque (Puskas *et al.* 2005). Calcium binding proteins mapped on CNP (Aho *et al.* 2005)
2006	CNP bind soluble palate, lung and nasal epithelium carcinoma-associated protein (SPLUNC), a lipopolysaccharide-binding host defence protein (Zhou *et al.* 2006). CNP cause renal calcifications after intravenous administration in rats (Shiekh *et al.* 2006). Anti-CNP antibody titre is a highly significant independent risk factor for coronary artery calcification (Ertas *et al.* 2006).

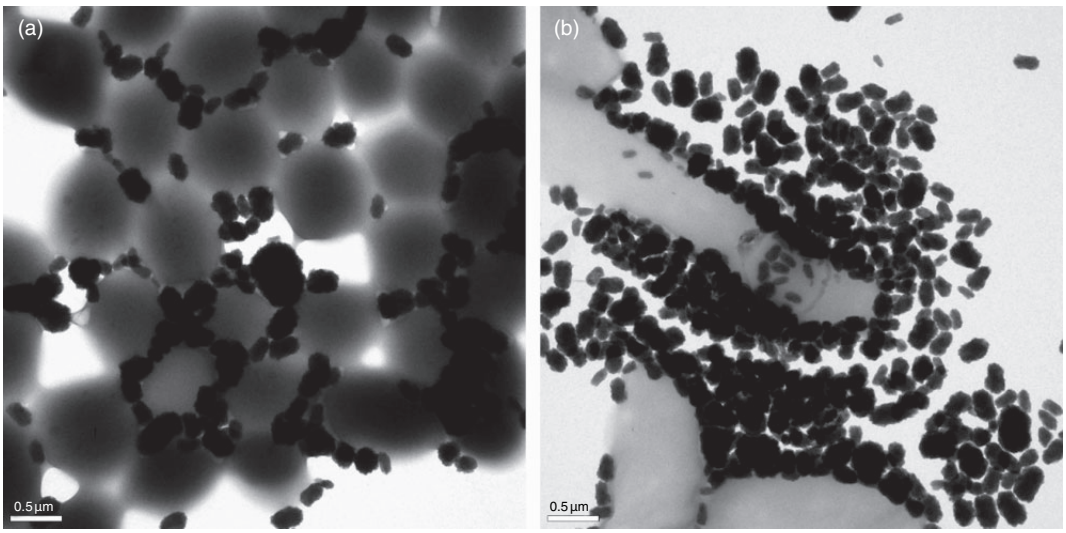

Figure 1 (a) TEM micrograph of *Agrobacterium tumefaciens*–calcifying nanoparticles (CNP) mixed biofilm after 7-h co-culture. Negative staining omitting the negative stain; thus the natural electron density gives the grey colour (*A. tumefaciens*) and black colour (CNP). Bar = 500 nm. (b) TEM micrograph of *Escherichia coli* DH5 alpha-nanobacteria mixed biofilm after 7-h co-culture. Negative staining omitting the negative stain; thus the natural electron density gives the grey colour (*E. coli*) and black colour (nanobacteria). Bar = 500 nm. Photograph by S.H. Harinen.

interactions that could involve changes in the normal microbial flora and pathogenic invaders, biofilms and parasitism. CNP are haematologic infectious agents, which can reach any part of the body. CNP grow better in the presence of other bacteria (Çiftçioglu and Kajander 2000). This could change the normal flora, and promote bacterial infection and bacterial biofilm formation. CNP are found in/on calcifications in the body, atherosclerotic plaque and outside the body surfaces (stones). Such calcifications are known to attract bacterial infections, e.g. endocarditis, valve and vascular infection, and infection of urinary and bile tracts. CNP adhere on foreign objects, such as implants, and could result in biofilm formation later attracting bacteria, leading to a chronic infectious problem that is often untreatable with antibiotics. CNP are known to participate in dental stone formation and could lead to periodontal disease in association with other bacteria (Çiftçioglu *et al.* 1998). CNP have been detected in tear fluids and salivary fluids. Little is known of their influence on microbial biofilm formation in the contact lenses, intraocular lenses and epithelium of cornea, and on the dental surfaces, prosthesis and dental implants. CNP are eliminated from body via bile and urine. It remains to be elucidated if their presence or absence could influence microbial flora in the gut and in the urinary tract. Further studies on CNP–bacteria interactions are warranted.

Conclusions and future prospects

Based on the current test results, CNP are not members of Eubacteria or Archaea, but form an entity of their own that may be of primordial origin (Sommer *et al.* 2003). At the moment, it is not enough to claim an agent not living according to the standard view on living creatures, as irrelevant to biological safety of cell cultures, or to human and animal health. Although the nature of prions is still under debate and prions are classified as nonliving, they exist and cause diseases, and thus form a serious risk for animal and human health. The risk was realized only after huge economical losses. It appears that CNP situation is rather similar, except the fact that CNP appear to cause or contribute to common diseases of the mankind.

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