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Nanobacteria Antigen and Antibody Titers in USA Controls Compared to UK Controls and Kidney Disease Patients

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INTRODUCTION

A broader appreciation of the role of microbes, their parts and toxins in acute and chronic human diseases has emerged in medicine in the 1990s [1-6]. Infections, opportunistic colonizations, toxicoses, molecular mimicry, unresolved cytokine-driven inflammation, and, perhaps, aspects of soft-tissue calcification [7] encountered in many human diseases are now part of disease etiologies. Nearly every chronic disease is now being investigated as being potentially microbe-initiated or promoted; as a result, sightings of 'new' microbes in human tissues has increased [8,9].

We focus here on nanobacteria, a 200-300 nm agent prone to surface calcification [10] that is reported to contain muramic acid, proteins, difficult to extract DNA, to incorporate nucleic acids into macromolecules [11-16], is positive for endotoxin in the differential Limulus Amebocyte Assay (LAL) and by anti-LPS antibodies [17], and growth stimulated by discrete wavelengths of light [18]. Nanobacteria were initially found to be culture resistant, but media and conditions now exist to propagate them *in vitro* [14, 16].

Described as nan(n)obacteria, nanoforms, ultramicrobacteria, nanovesicles and other names, their existence has been reproduced in several laboratories by scientists of diverse disciplines. Astrobiology, geology, environmental sciences, biology, and medicine have been touched by sightings and discussions of these particles.

What, if any, is the role of small (<0.3microns) self-replicating particles in nature and medicine? The similarities and differences between/among these particles continue to be debated. Are they alive or chemically-active but not alive, or tiny dormant (perhaps spore-like) forms of more classical microbes, or remnants of known microbes or cells, or precursors of life on earth... [18]. Alternative interpretations of nanobacteria etiology include self-propagation of phospholipid-protein-calcium phosphate aggregates [19], prion-like calcium-binding proteins, and diverse biomineralizations involving remnants of calcium binding molecules [21].

Findings consistent with a role for such particles in human and animal diseases include:

- Association of nanobacteria or its putative antigens at sites of active disease: kidney stones [20,21], kidney cysts and pineal cysts [17,22,23], atherosclerotic lesions in carotid artery and aorta [24], dental stones [13,25], calciphylaxis [unpublished]; particles with similar morphology were reported in CSF from patients with schizophrenia [26].
- Nil to high cytotoxicity *in vitro* against mammalian cells; the level of toxicity was dependent on the isolate; human- and animal-derived isolates were used [17,27].

- Susceptibility in vitro (i.e., cidal or static effects) to an array of drugs (e.g., tetracycline, 5-FU; both cidal) with and without known dictating activities [28].
- Immuno-cross-reactivity with *Bartonella* sp. and LPS of Chlamydial, both organisms are human pathogens [17,27].

In this preliminary study, we ask '**do nanobacteria elicit an immune response in humans**' and '**does such a response correlate with active disease, carriage, or colonization**'?

We measured human sera for the presence of a) nanobacterial antigen(s) as a marker for the presence of nanobacteria AND b) human antibodies made against nanobacteria as a marker for an immune response to nanobacteria.

ABSTRACT

Nanobacteria (NB) are newly discovered microbial agents that are unique due to small size (0.2-0.5nm) and a calcium apatite shell. NB are hypothesized to be the cause of extra-skeletal calcifications e.g. kidney stones, atherosclerotic lesions and renal cysts. NB-associated biogenic apatite is both antigenic and immunogenic; calcium apatite is not.

Objectives: In this initial survey, sera from 22 patients with various chronic renal diseases (UK-P) and controls (blood donors) from the UK (20; UK-C) and USA (20; USA-C) were tested for antibody (Ab) and antigen (Ag) to NB.

Methods: ELISA kits were used (Nanobac Oy, Kuopio, FI). For this study, unit values of Ab and/or Ag 1.0 were considered positive. Samples for Ab were diluted 1:500; for Ag, 1:5 and 1:10 due to prozone-like effect observed at recommended 1:1 dilution.

Results: NB Ab positivity was >2.5-fold in UK-C (35%) than USA-C (14%) and >2-fold than renal patients (18%). NB Ag positivity was similar for the three groups (mean 63%; range 59-69). Ag/Ab titers were examined for various permutations of positivity (see Table). NB Ab or Ag positivity did not correlate with age (range: 23-68 years) or gender.

Table:

Ab	Ag	UK-C	USA-C	UK-P
+	+	20%	5%	9%
+	--	15%	9%	9%
--	+	40%	64%	50%
--	--	25%	22%	32%

Conclusion: This initial study for NB Ab and Ag in presumed healthy individuals in Scotland and Central Illinois revealed a >2 fold difference in positivity. The relationship between NB positivity and acute/chronic human disease(s) involving soft tissue calcification is unknown. Interestingly Scotland has a higher rate of cardiovascular (i.e., calcified plaque) disease man USA. Kidney patients had lower NB positivity perhaps due to being on haemodialysis, thus clearing NB more effectively. Expanded studies are warranted to determine precise breakpoints for Ag/Ab positivity and negativity in health and various disease states.

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