



Potential role of calcifying nanoparticles in the etiology of multiple sclerosis

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ABSTRACT

Nanobacteria or calcifying nanoparticles are 80–500 nm sized nano-organisms that are physically associated with carbonate apatite mineral formations. They have been indicated in various diseases, including kidney stone formation, Alzheimer's disease, and atherosclerosis. Nanoparticles contain calcium and apatite-binding protein fetuin-A, a calcification inhibitor. However, recent evidence indicates that fetuin-A can form nucleation seeds or nidi that grow in size through ion sedimentation to become larger amorphous nanoparticles in the presence of excess calcium and apatite ions.

Fetuin-A also functions as an inhibitor of meprin, a metalloproteinase implicated in inflammation and neurodegenerative diseases. During inflammation, meprin functions to regulate chemokine activity of monocyte chemoattractant protein 1, which is associated with chronic inflammatory diseases, including atherosclerosis, renal inflammatory diseases, and multiple sclerosis (MS). In addition, calcium phosphate nanocrystals that contain fetuin-A are pro-inflammatory to macrophages and promote vascular smooth muscle cell mineralization, potentiating a vicious cycle of inflammation and calcification. Thus, mineral stress and inflammation appear to be associated with each other. Furthermore, fetuin-A deficient mice exhibited reduced experimental autoimmune encephalomyelitis severity. Thus, fetuin-A plays a direct role in the neuroinflammatory response. Indeed, the level of fetuin-A in cerebrospinal fluid has been defined as a biomarker of disease activity in MS.

MS is a chronic, inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS) with an unknown etiology. The “inside-out” model of MS, supported by recent data, states that the initial axonal degeneration in the CNS occurs before demyelination, which then stimulates an auto-immune attack. It was shown very recently that influx of calcium from the extracellular space through nanoscale ruptures of the axonal plasma membrane predict axon degeneration in neuroinflammation. Calcium is an activator of calpains, proteases that function to break down the cytoskeleton, leading to neurodegeneration. Nanoruptures of the plasma membrane were suggested to occur at the early stages of axon damage, especially at nodes of Ranvier, which are devoid of myelin.

Here, I propose that calcifying nanoparticles may have a role in the etiology and/or pathophysiology of MS. The initial event causing neurodegeneration may be due to the nanoparticles that have been suggested to easily cross the blood-brain barrier. Following this, the nanoparticles may create nanoruptures in the axonal membrane and also increase the calcium concentration around and within the neurons by forming nidi for calcification, eventually causing neurodegeneration. Nanoparticles can self-replicate; hence, they may represent an infectious causative agent for the development of MS.

Introduction

Nanobacteria, also known as nanons, calciprotein particles, or calcifying nanoparticles, are bacteria-like organisms that were initially observed more than 30 years ago in blood and blood products as species remarkably resistant to gamma irradiation and heat. Nanoparticles range in size from 80 to 500 nm, are coccoid in shape, and can be filtered through 100 nm-pore filters [1].

In scanning electron microscopy, nanobacteria have been observed to be associated with apatite mineral formations, which presumably are

the cause of nanobacterial resistance to extreme changes in ambient conditions. The mineral coating surrounding nanobacteria was identified to be carbonate apatite, as found in the teeth and bones of humans [2]. In addition to electron microscopy, nanobacteria can be detected with specific monoclonal antibodies and cell culture techniques. Nucleic acids cannot be directly isolated from nanobacteria, probably due to their mineral coating. Even though 16S rDNA sequences for *Nanobacterium sanguineum* and *Nanobacterium* sp. were registered in NCBI GenBank, these sequences were later determined to be indistinguishable from those of a contaminating microorganism [3].

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These observations have led some researchers to consider nanobacteria as unusual forms of crystals rather than as organisms [4]. In this view, nonliving mineralo-organic nanoparticles could be cell precursors or primordial cells and could be considered as nano-sized particles, much like lipoproteins, ferritin, and viruses [2]. Nevertheless, these nanoparticles are self-replicating and can be cultured [4].

Nanoparticles and diseases

There is evidence showing that nanoparticles may contribute to various diseases and degenerative processes, including kidney stone formation, polycystic kidney disease, gallstones and gallbladder inflammation, prostatitis, calciphylaxis, and ovarian cancer. On being detected in dental calculus, they were also believed to be involved in the pathogenesis of periodontitis [4].

Moreover, ectopic calcification found in soft tissue, such as the one occurring at the site of an atherosclerotic plaque in coronary arteries and leading to arterial heart disease, is claimed to be due to the action of calcifying nanoparticles [4]. A clinical trial investigating anti-nanobacteria treatment against arterial calcification has demonstrated a reduction in plaque formation and regression of plaques following therapy [2]. These findings require confirmation in larger clinical trials. Furthermore, nanoparticles have been suggested to be associated with the amyloid accumulations of Alzheimer's disease [4].

Nanoparticles contain only serum proteins, e.g. fetuin-A (alpha-2-HS-glycoprotein) and albumin, and are described as mineralo-fetuin complexes. Calcium and apatite-binding proteins, albumin and fetuin-A, are known as calcification inhibitors that form amorphous, soluble mineral nanoparticles, which function to prevent ectopic calcification and therefore prevent systemic mineral crystallization in the body. Conversely, it has been suggested that when these calcification inhibitors are outnumbered by calcium and apatite ions, they form mineralo-organic foci or nucleation seeds (nidi) that grow in size to become larger amorphous nanoparticles via further ion sedimentation as well as conformational and phase changes. Eventually, the nidi progress to full crystallization [2]. Thus, fetuin-A has been called a mineral chaperone, carrying excess calcium and apatite in the circulatory system to ectopic locations for soft tissue mineralization [5].

Fetuin-A

Fetuin-A is a serum protein synthesized mainly by hepatocytes and has been connected to a wide variety of physiological and pathological processes related to bone and to mineralization, metabolism, cardiovascular system, and the central nervous system (CNS) [6].

For example, fetuin-A has been suggested to function as an inhibitor of meprin, a metalloproteinase (Mori et al. 2011) implicated in inflammation, fibrosis, neurodegenerative diseases, angiogenesis, and cancer [7]. Meprin functions in the regulation of monocyte chemotactic protein 1 (MCP-1/the chemokine CC motif ligand 2 or CCL2) chemokine activity during inflammation. CCL2 has important roles associated with chronic inflammatory diseases, including asthma, atherosclerosis, rheumatoid arthritis, renal inflammatory diseases, and multiple sclerosis (MS) [8]. Fetuin-A containing calcium phosphate nanocrystals have been found to be pro-inflammatory to macrophages and promote vascular smooth muscle cell mineralization, potentiating a vicious cycle of inflammation and calcification. Thus, mineral stress and inflammation seem to be associated with each other [9]. Furthermore, fetuin-A deficient mice were observed to exhibit reduced experimental autoimmune encephalomyelitis (EAE) severity, demonstrating that fetuin-A plays a direct role in the neuroinflammatory response in the mouse EAE model [10].

Hence, fetuin-A in the cerebrospinal fluid (CSF) is considered as a biomarker of disease activity in MS. Significantly increased CSF fetuin-A levels that decrease to baseline levels upon treatment with natalizumab were observed in MS patients with active disease. Moreover,

fetuin-A levels were related to an increased risk of conversion from a clinically isolated syndrome to a relapsing-remitting MS [10].

The cause of multiple sclerosis is unknown

MS is a chronic inflammatory demyelinating and neurodegenerative disease of the CNS and is one of the leading causes of non-traumatic neurological disability in young adults. The etiology of MS is currently unknown, however, some genetic and environmental risk factors may play a role in its development. The most commonly implicated environmental risk factors include Epstein-Barr virus infection, cigarette smoking, vitamin D deficiency, obesity, and microbiota.

There are currently two models to explain the development of lesions in MS. For many years it was believed that demyelination happens before axonal damage and loss, as the so called “outside-in” model states. However, the “inside-out” model of MS, which is supported by recent data, states that the initial axonal degeneration in the CNS occurs before demyelination and subsequently stimulates an auto-immune attack.

Very recently, *in vivo* calcium imaging in an MS model (EAE) has shown that cytoplasmic calcium accumulations predict axon degeneration in neuroinflammation. Calcium is a multi-functional second messenger acting in the signal transduction pathways of several hormones and neurotransmitters and functions in the regulation of inflammation and immunologic responses. In particular, calcium is an activator of some proteases, such as calpains, that function to break down the cytoskeleton, leading to neurodegeneration. By ruling out other sources of intra-axonal calcium, the influx of calcium from the extracellular space was identified as a driver of axon degeneration. Nanoscale ruptures or disruptions of the axonal plasma membrane, which have been previously described to occur in response to traumatic CNS injuries, were found to allow entry of calcium. Nanoruptures of the plasma membrane were suggested to occur at the early stages of axon damage, especially at areas devoid of myelin, i.e. the nodes of Ranvier [11].

Hypothesis

Here, I propose that calcifying nanobacteria or nanoparticles may have a role in the etiology and/or pathophysiology of MS due to their coexistence with fetuin-A, a biomarker of MS. It has been suggested that nanoparticles can easily cross the blood-brain barrier. Particles larger than 10 nm, such as antibodies, cannot pass the blood-brain barrier. Nanoparticles are much larger than this, however, their association with phospholipids may render them lipid-soluble and therefore facilitate their entrance to the CNS. Therefore, the initial event causing neurodegeneration may be due to calcifying nanoparticles and may, thus, be a candidate etiological factor in MS. Moreover, nanoparticles may have dual roles in the pathophysiology of MS. They may first pass the blood-brain barrier, create nanoruptures of the axonal membrane, increase the calcium concentration around and within the neurons by forming nidi for calcification, and eventually cause neurodegeneration. Finally, the breakdown of the blood-brain barrier enables entry of T-lymphocytes to the CNS.

Consequences of the hypothesis

Nanoparticles can grow and self-replicate like bacteria; hence, they may represent an infectious causative agent for the development of MS. Future research in MS, nanomedicine, and nanotoxicology is needed to investigate the role of nanoparticles in MS pathophysiology, as well as their relationship with the recognized risk factors of MS, including Epstein-Barr virus and toxic chemicals present in cigarette smoke.

Research in this field will improve our understanding of this potentially disabling disease, hence, pave the way for new treatment strategies, or better, its eradication.

Source of support

None.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.05.005>.

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