

Fetal Bovine Serum and Nanobacteria

Fetal Bovine Serum - **Varicella Vax, some oral polio vax, Merck reported it was in MMR** to Ray Gallup , and in the former **Rotavirus** vaccine.....

"As reported May 23rd, 2001 at the 101st General Meeting of the American Society for Microbiology, Nanobacteria has been found to be a contaminant in previously assumed-to-be-sterile medical products, specifically **IPV Polio Vaccine**. Most human biologicals and vaccines are made in fetal bovine serum, a medium that is known to be contaminated with nanobacteria. In order to prevent this problem in the future, human biological products must be made in Nano-Free Culture medium (filtered first through 20 nanometer filters, Gamma-Irradiated with 150 megarads and then heated to 90 degrees Centigrade for at least an hour to kill any nanobacteria present)"

excerpts.....

"The term "Nanobacteria" is short for it's scientific genus & species name "Nanobacterium sanguineum", a Latin scientific term for blood nanobacteria. Nanobacteria are "nano"-sized in that they are from 20-200 nanometers in size (a nanometer is 1 billionth of a meter. A nanometer is the width of ten hydrogen atoms side-to-side!) and are the smallest known self-replicating bacteria. They are from the Archaea Family of bacteria, known for their primitive pleomorphic lifestyles"

"Nanobacteria infection by Nanobacterium sanguineum is an "emerging infectious disease" meaning that it is newly discovered and that the diseases it cause are being researched and further described. Its DNA, RNA and Lipopolysaccharide profiles have been accurately mapped by multiple scientific researchers at many universities worldwide. Nanobacteria are not nice bugs and have absolutely no known positive benefits to humans. The discoverers of nanobacteria, Drs. Ciftcioglu & Kajander developed antigen & antibody diagnostic blood testing for nanobacterial infections that we offer as the "NanobacTEST". NanobacLabs has developed safe and effective nanobiotic prescription treatments"

"Nanobacteria are extremely small, slowly growing bacteria that can be cultured from the blood of humans and mammals. Their size is 20-200 nanometers....when compared to "regular" bacteria, Nanobacteria are 1/100 to 1/1,000 the size, allowing them to easily move around into other cells and invade them. Nanobacteria cause apoptosis (cell death) when exposed to human cells or other bacteria. They can cause alteration of RNA and DNA gene-expression patterns of cells they infect.....this can lead to genetic alteration, abnormal cell growth and proliferation. When compared to other bacteria, Nanobacteria grow very, very slowly, only reproducing every 3 days.....where "regular" bacteria reproduce in minutes or hours. Nanobacteria cannot be grown in standard culture media and can only be grown in mammalian blood or serum.

Nanobacteria were discovered in 1988 by Nobel Prize Nominees Dr. Neva Ciftcioglu, PhD and Olavi Kajander, MD, PhD as a "contaminant" in mammalian cell cultures that kept killing the mammalian cells (apoptosis) in their mammalian cell culture research. They have been researching nanobacterial pathophysiology for nearly 14 years now and are the worldwide experts on nanobacterial basic science. They are currently guiding and teaching researchers all over the world. NanobacLabs is the world leader in the research and development of prescription NANOBOTICS that eradicate pathological calcification and nanobacterial infections"

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bovine serum, a medium that is known to be contaminated with nanobacteria. In order to prevent this problem in the future, human biological products must be made in Nano-Free Culture medium (filtered first through 20 nanometer filters, Gamma-Irradiated with 150 megarads and then heated to 90 degrees Centigrade for at least an hour to kill any nanobacteria present)"

A new potential threat for blood and blood products, cell culture, cell and tissue banking and biotechnology has been discovered: *Nanobacterium sanguineum* gen. et sp. nov.. These self-replicating ultrafilterable bacteria were isolated from over 80% of commercial "sterile" fetal and newborn bovine sera and are thus the most common contaminant present in cell cultures. Growth occurred in vitro under cell culture conditions (with or without mammalian cells) but not under anaerobic conditions. Their doubling time was 1-5 days. They were culturable also in protein and lipid-free medium beyond three years with monthly passages. Colony formation on solid media was poor. The agent multiplied in culture with serum in a logarithmic mode that could be prevented with aminoglycoside antibiotics, EDTA, cytosine arabinoside and gamma irradiation. They showed procaryotic structure with specific antigens detectable by monoclonal antibodies, were generally mobile, coccoid with a diameter of 200 to 300 nm in serum, stained poorly with bacteriological stains, were very resistant to antibiotics and passed through 100 but not 50 nm filters. Their aminoterminal protein sequences were novel and reproducible. Considerable evidence suggested presence of nontraditional DNA. They incorporated radiolabelled uridine into DNA. 16S rRNA gene sequence results place them in alpha-2 subgroup of Proteobacteria which includes *Brucella*, also pathogens of mammals with preference to the fetus. This new agent causes cytotoxicity and senescence in many cultured cell lines by apoptotic cell death and growth arrest.

Several vaccines are currently being produced by using cultured mammalian cells. Microbiological sterility of such vaccines is of great importance since several examples indicate potential safety hazards in vaccines contaminated with unknown organisms. Fetal bovine serum (FBS) used as a supplement in cell culture is a known safety risk (Hodgson, 1995). Obviously, not all of the risk factors of FBS are yet known and thus cannot be controlled. It is commonly known that only about 10% of FBS batches support cell cloning well (Liddel and Cryer, 1991) but the reasons for this have remained unclear. As with many other cell cultures, we faced a problem about 10 years ago of poorly thriving cells not attributable to any known contaminant. In this report, we describe the discovery of a new bacterium from mammalian blood and blood products, tentatively named as *Nanobacterium sanguineum* gen. et sp. nov., and show that this agent is common and harmful.

DISCUSSION

Culture and Diagnosis of Nanobacteria The discovery of Nanobacteria came about because we had a problem with cell cultures namely vacuolized cells (Fig. 1A) and poorly thriving cultures without any contaminant detectable by standard methods. Transmission electron microscopy (TEM) made from these poorly thriving cell cultures indicated the presence of internalized procaryotic organisms (Fig. 1B). That their source was the commercial "sterile" FBS was proven by gamma-irradiating all the culture components (Table 1). This experiment also indicated that sterile culture media for detection of new organisms can be made by using gamma-irradiated serum as a supplement. The new organisms passed through 100 nm (but not 50 nm) filters and were called nanobacteria, since no other bacteria are known that can pass through filters with such small pores. This ability to pass through such small-pore filters was most remarkable since they were shown to have a cell wall and yet were able to surpass the filterability of cell-wall-less bacteria. They were unculturable in microbiological media but could be cultured under cell culture conditions (with or without mammalian cells, CO₂ 5-10%). These minute generally coccoid organisms had a diameter of 200 to 300 nm in serum, and their size increased during the culture due to the production of a very thick cell envelope (Fig. 1C, D). The thick and calcified envelope made them visible even by light microscopy. The doubling time of nanobacteria was 1-5 days

(Fig. 2). Their multiplication could be detected by specific ELISA, optical density, microscopic counting, SDS-PAGE or methionine and uridine incorporation, and the multiplication could be prevented with high doses of aminoglycoside antibiotics, EDTA, cytosine arabinoside and gamma-irradiation. Considerable evidence suggested the presence of nontraditional DNA. 16S rRNA gene sequence results (data will be published elsewhere) placed them into the alpha-2 subgroup of Proteobacteria which includes Brucella (which are also pathogens of mammals with preference to the fetus) and Bartonella.

Nanobacteria were isolated from more than 80% of commercial FBS and newborn bovine sera and are the most common contaminant present in cell cultures. In addition, we isolated nanobacteria from the blood of 4% of medical students at our university. Positive identification of nanobacteria involved growth in cell culture medium with typical growth rate and optical properties, specific stainability with Hoechst 33258 using the high dye concentration and positive immunoassay results with immunofluorescence and/or ELISA using monoclonal anti-nanobacteria antibodies.

Cytotoxicity of Nanobacteria Nanobacteria are cytopathic in cell cultures and invade mammalian cells in a distinctive manner: They trigger cells that are not normally phagocytic to engulf them. These novel organisms are one of the causes for cell vacuolization, poor thriving and unexpected cell lysis, problems often encountered in mammalian cell culture. Several mammalian fibroblast lines were cultured in MEM medium as described previously (Kajander et al., 1990), and were infected with nanobacteria. Electron microscopy and FITC staining with specific monoclonal antibodies indicated that nanobacteria were bound on the surface of the fibroblasts (Fig. 1E-G). We concluded that they were internalized either by receptor-mediated endocytosis or by a closely related pathway. After the internalization, fibroblasts showed apoptotic abnormalities and died if subjected to a high dose (>100 nanobacteria/cell).

Different Growth Phases of Nanobacteria Washed nanobacteria added to serum-free medium grew very slowly as evidenced by increase in their numbers and protein level and were firmly attached to the culture plates. These cultures progressed to large multicellular formations covered by layers of a firm protective material several micrometers thick (Fig. 1H). After addition of sterile serum, the layer disappeared, with typical small coccoid nanobacteria later appearing in the same cultures with the mobile, larger D-shaped ones (Fig. 1I). Specific monoclonal antibodies indicated the presence of the same antigenic sites in both D-shaped and coccoid nanobacteria, and their 16S rRNA gene sequences were 98% identical.

How can Cell Culture be Possible with Nanobacteria-contaminated Fetal Bovine Serum? Although more than 80% of cell culture serum batches are contaminated with nanobacteria, many cell culturists have not faced this problem with their cell cultures. We have experienced a major problem with nanobacteria in cell culture only when they are present at high concentrations relative to cells. This can occur typically in cell cloning and in long-term experiments where mammalian cells do not multiply. Internalization of numerous nanobacteria by a cell results in cytotoxicity. Importantly, most cell lines multiply faster than nanobacteria. Thus, cytotoxic concentrations may be avoided.

Why is Nanobacteria a Potential Threat? Nanobacteria can cause a chronic infection in laboratory animals and in humans. The agent could be isolated from blood of one person for 5 years despite the presence of antibody. When nanobacteria were injected into rabbits, the agent was initially isolated from urine and then from cerebrospinal fluid after one year. Nanobacteria multiply very slowly and, if pathogenic in humans, might cause slow chronic autoimmune-like disorders (compare with leprosy or brucellosis). So far, there are no chronic bacteraemia known that would not be harmful. Thus, the possibility that nanobacteria may be present in vaccines made with cell culture, or in gammaglobulin or other antibody preparations, must be controlled.

SUMMARY AND CONCLUSIONS Nanobacteria are novel microorganisms that are not detectable with

present sterility testing methods, but they are detectable with new culture and immunomethods. They are commonly present in bovine and blood products and thus in cell cultures and antigens, including vaccines derived therefrom, and may be present in antibody and gammaglobulin products. Nanobacteria are a potential risk because of their cytotoxic properties and ability to infect fetuses, and thus their pathogenicity should be scrutinized.

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Figure 1. Ultrastructure of nanobacteria and their interaction with fibroblasts.

(A) Perinuclear vacuolization of an infected 3T6 cell under phase-contrast microscope;

(B) TEM image of a nanobacterium engulfed by a BHK cell;

(C) cultured coccoid nanobacteria (bars 200 nm).

(D) SEM image of nanobacteria attached to culture vessel;

(E) nanobacteria attached to a fibroblast surface (arrow shows the surface of the fibroblast; bars 1 μ m).

(F) Indirect immunofluorescence staining of cultured healthy 3T6 cells with a monoclonal antibody (8/0) against nanobacteria;

(G) 3T6 cells inoculated with nanobacteria;

(H) TEM of a nanobacterial population in a serum-free culture (arrow shows a D-shaped nanobacterium in this population);

(I) D-shaped nanobacteria after culture in serum-containing medium (bars 1 μ m).

Figure 2. Growth-curve of nanobacteria. As a control, gamma-irradiated FBS was used. At each time point, samples from triplicate incubations were taken, frozen and analyzed by turbidometer at the end of the experiment. Turbidometer units are means of three measurements from 1/6 dilutions of cultures.

Table 1. The Effect of ⁶⁰Co Gamma-Irradiation of Culture Components on Multiplication of Nanobacteria

Culture Multiplication

FBS

RPMI +

FBS

*RPMI +

*FBS

RPMI -

*FBS

RPMI
nanobacteria or FBS +
*FBS
RPMI
*nanobacteria or * FBS

The material marked with asterisk (*) received a sterilization dose of 3 megarads during 16 h at room temperature. Cultures were established using 10 % serum and nanobacterial counts were followed for 4 weeks.

"Another intriguing subject is that of the putative nanobacteria studied by a Finnish group. Present in human and bovine sera, they might have contaminated many biological preparations and have spread in human populations. As they induce deposition of calcium salts, they may be involved in diseases involving such depositions, such as renal lithiasis and atherosclerotic plaques, or even neuro-degenerative diseases. Their minimal size (200 nM) precludes conventional packaging of a length of DNA sufficient to code for an autonomous microorganism. But it is possible that their genetic information is encoded in a modified, more compact DNA. In conclusion, our fight against emerging diseases has just begun. We should always be vigilant against the resurgence of known infectious germs and the emergence of new agents. More than ever, a world-wide network of sentinel laboratories and a coordinated multidisciplinary effort in biomedical research are required for our survival. "