Nanobacteria and associated ‘elementary bodies’ in human disease and cancer

Reports showing that very small bacteria can be isolated from environmental samples and human blood have recently caused considerable excitement and controversy. Called nanobacteria (or nanobacteria), these very small bacteria appear as spheres and ellipses of a diameter between 0·03 and 0·2 µm, often occurring in chains or groups of similar-sized forms (1). Nanobacteria have been isolated from blood as clusters of coccus cell-walled organisms (0·08–0·5 µm) and associated ‘elementary particles’ (0·005–1 µm) which together produce biofilms containing carbonate or hydroxypatite. Recent data from 16S rRNA gene sequences have positioned blood-borne nanobacteria in the α-2 subgroup of the Proteobacteria (2). Such isolates are extremely resistant to heat and certain antibiotics, and exhibit a ‘bizarre morphology’ (i.e. extreme pleomorphism).

Although nanobacteria are usually portrayed as being novel, very small bacteria have frequently been reported in the past and have been associated with a wide variety of diseases, notably cancer (3, 4, 5). Very small entities, similar to elementary particles of nanobacteria or the ‘elementary bodies’ (6) of species of Chlamydia and mycoplasmas (mollicutes) have also frequently been mentioned in the historical literature under a confusing variety of names, including elementary forms, gonidia, granules, inclusion bodies, infrabacteria, arthrobacteria and antebacterial forms.

Ultra-small bacteria and elementary bodies were for a long time regarded as part of the bacterial life cycle and associated with extreme bacterial pleomorphism (4, 5). For example, Bechamp, a contemporary and rival of Pasteur, claimed to have found so-called ‘microzymas’ in the body, i.e. very small entities, capable of independent existence. In 1873, Lister found minute granules in urine which grew by dividing into four units (so-called ‘fissiparous generation’) and which he termed ‘Granuligera’ (8). Belief in the existence of such elementary bodies continued with Enderlein who claimed that blood cells contain primitive life forms which he termed ‘protits’. Such protits were seen under dark-field illumination and were of the order of 0·01 µm in diameter. Gaston Naessens continued this tradition with the ‘somatid’, an elementary particle that apparently survived the death of the infected organism and then regenerated into bacteria, often via a complex life cycle (7).

During the early part of this century a number of microbiologists claimed that bacteria could pass through ultra-fine filters and then be regenerated as normal-sized bacteria on cell-free media. Kendall and Hadley, the main advocates of bacterial filterability claimed that disease-causing bacteria, or a phase of their growth cycle, could pass through filters (7). Filter-passing bacteria were originally referred to as ‘viruses’, a confusing term first used to refer to any infective agent, then to filter-passing bacteria, finally achieving its modern definition following the appearance in 1928 of Rivers’ seminal book Filterable Viruses (9). Gruner (10), quoting Lipshutz, commented that filterable bacteria are part of the bacterial life cycle and are unable to grow except when in symbiotic association with a septic organism. Such ‘filterable bodies’ (0·2 µm and smaller) have frequently been isolated from patients suffering from the wide variety of diseases, including the common cold, herpes, influenza, meningitis and smallpox (11).

It could be argued that this historical literature can be ignored because it was based on microscopy and isolation techniques that have been superseded by modern molecular approaches. Some of these studies were subjected to a variety of contemporary criticism; so-called ‘bacterial life cycles’ were, for example, thought to result from contamination and the fanciful linking of individual forms into a non-existent cycle. Other critics suggested that pleomorphic bacterial forms, including ultra-small bacteria, resulted from staining or chemically induced artefacts; many of these criticisms were, however, countered by the claimants (4). Despite such criticism, evidence supporting the existence of ultra-small bacteria has been accumulating for over a century, and has been backed up by the recent application of modern molecular techniques.

The historical literature on filterable bacteria and elementary bodies finds its modern equivalent in the work of Domingue & Schlegel (12) who found that when filter-passing bacteria (0·2 µm) were grown on laboratory media they reverted to normal-sized bacteria. They also noted the appearance of what they called ‘small dense bodies’ which were observed microscopically, but disappeared when ordinary bacteria grew; a few of these bodies were shown to revert to normal bacteria. Domingue & Woody (13) have also reported what they term as...
elementary reductive units (ERU) which are apparently part of a complex pleomorophic life cycle of cell-wall-deficient bacteria. Similarly, Kajander et al. have reported (2) the presence of elementary particles (0·05–0·1 µm) in filtered samples of human blood. These are also large enough in size to allow cells that can replicate might release smaller particles which cannot multiply on their own. Itoh et al. (14) also isolated granular bodies after passing *Spiruuspora minuta* (the sucking mouse cataract agent) through a 0·2 µm filter. When cultured, these gave rise to helical filaments on which small granular bodies appeared. As the culture aged, larger ‘sphercic bodies’ containing ‘granular bodies’ appeared; the nature of these latter bodies was not determined. So-called filterable ‘granular cocci’ have also been isolated from commercially prepared BCG vaccines (15). Although the smallest of these may be incapable of independent existence, they can apparently aggregate together to form viable units.

A considerable literature exists implicating highly pleomorphic bacteria in cancer (16, 17, 18, 19). Extremely small particles, approximating to elementary bodies, have also frequently been reported as part of the life cycle of ‘cancer germs’ (5, 10, 17). The nature of these latter bodies was not determined. So-called filterable ‘granular cocci’ have also been isolated from human blood (2). For example, recently Diller & Donelly (24) isolated a pleomorphic bacterium from rat and mouse tumours, and in mycobacteria from human carcinomas, from the blood of hundreds of cancer patients and leukaemic blood. These authors claim that larger nanobacteria (0·1 µm) in filtered samples of human blood.

**Milton Wainwright**

Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, S50 2TN.

Tel: +44 114 222 4410. Fax: +44 114 272 8697.

e-mail: m.wainwright@sheffield.ac.uk