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Current Developments in Vaccines against Meningococcal Disease

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The meningococcus and the need for a vaccine

It is nearly two centuries since Vieusseaux described the first outbreak of a "cerebrospinal fever"[1], and over a century since Weichselbaum[2] discovered the meningococcus, which was first named *Diplococcus intercellularis meningitidis*.

Neisseria meningitidis, a fastidious Gram-negative and oxidase positive diplococcus, is an obligate human pathogen and its usual relationship with the host is one of commensalism. However, a minority of the exposed individuals will develop one of a variety of clinical syndromes, ranging in severity from a transient mild sore throat to meningitis and to septicaemic shock which can kill within hours of the appearance of symptoms (**Figure 1**). Despite the sensitivity of the organism to many antibiotics and despite the high standard of health care, mortality from meningococcal disease remains relatively high in Europe and North America (5-14% from meningitis and over 50% from severe septicaemia). A proportion of the patients who recover end up with permanent neurological sequeleae. In developing countries where over 0.3 million people are thought to suffer from meningococcal diseases annually, morbidity and mortality from meningococcal disease is much greater than Western countries. In countries within the "meningitis belt" of savanna Africa, attack rates can reach as high as 1,000 cases per 10^5 population, which contrasts with 5-25 cases per 10^5 in the industrialised countries. Therefore, prevention of the disease with vaccines is a global priority.

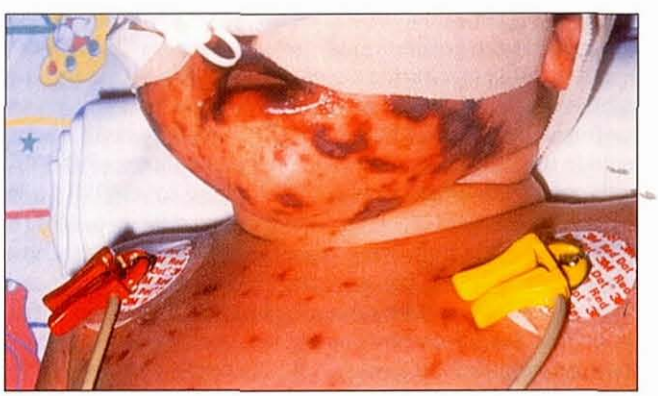

Figure1. Meningococcal septicaemic shock in a young child, admitted to the Nottingham Intensive Care Unit. Note the extensive haemorrhagic rash and generalised oedema.

An ideal meningococcal vaccine should be safe, protect against all meningococci, be given orally or nasally, offer long-lasting immunity to all age groups, and be easily incorporated into the World Health Organisation's Expanded Program on Immunisation. So far no such vaccine has been developed. Nevertheless, capsular polysaccharide (CPS)-based vaccines have been developed against some but not all meningococcal serogroups. However, these suffer major limitations and the search is going on for suitable alternatives.

Despite extensive studies, the mechanisms responsible for the development of natural immunity against meningococci remain unclear. Protection has been correlated with the presence of bactericidal antibodies, hence the focus of the great majority of the studies on this arm of the host defence. Much less attention has been given to the killing of bacteria by phagocytosis and, therefore, little is known about the relative importance of phagocyte-mediated killing of meningococci as compared to serum bactericidal activity. A close association has been found between the opsonin activity, duration and severity of symptoms and the level of anti-outer membrane

Also in this issue (page 5):

The Role of the *Bacillus* genus in Infection

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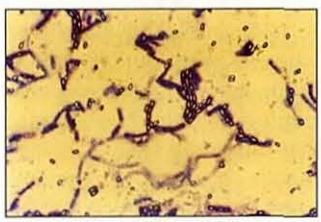
A comprehensive and up to date review of the *Bacillus* genus in Infection.



proteins IgG[3]. Moreover, for most antigens, an efficient humoral immune response resulting in the production of antibodies and the generation of memory response requires help from T-lymphocytes. However, T-cells respond to peptide antigens associated with molecules of the major histocompatibility complex and so will not be stimulated by polysaccharide vaccines, which explains why the protective efficacy of the currently available CPS-based vaccines is short lived and ineffective in young children (see below).

Capsular polysaccharide vaccines

Serogroup A and C vaccines

The first successful vaccines against Group A and C meningococcal strains were developed in the late 1960's when Cetavlon-precipitated high molecular weight (>100 kDa) CPSs were found to be protective in small-scale field trials in military recruits. These were followed by a series of larger field trials among different age groups in different parts of the world, including Europe, Africa and Latin America. These trials showed that antibody responses to the serogroup A and C polysaccharide vaccines depended on a number of factors, including the age of the vaccinee. It was clear that the serogroup A vaccines were effective in controlling epidemics of serogroup A disease and are, therefore, recommended to be given during epidemics to all age groups including infants, with a booster dose offered to those under the age of 18 months. Serogroup C capsular vaccines, however, are now recommended for general use in epidemics but not for children under the age of 18 months. These vaccines are serogroup-specific (therefore, ineffective against serogroup B), their protection lasts no more than three years, and offer little protection to children under the age of two years. Therefore, they are not useful for routine immunisation of infants.

The CPS of *Haemophilus influenzae* type b (Hib) is also a poor immunogen in infants and the original CPS-based Hib vaccines were of limited use. However, by covalently linking the CPS to protein carriers, vaccines which give excellent protective responses in infants have been developed. This success has led to the inclusion of such vaccines in the routine childhood immunisation program of many countries, including the United Kingdom. Hib vaccine induces a T-cell dependent IgG response in young children, and thus immunological memory. This has encouraged the development of CPS-protein conjugate vaccines for serogroups A and C meningococci. The World Health Organisation's Steering Committee on Encapsulated Bacteria Program for Vaccine Development, has been coordinating and facilitating the development of conjugate vaccines against *N. meningitidis*, particularly serogroup A, and has encouraged manufacturers to produce conjugates for evaluation.

Group A and C capsules conjugated to non-toxic mutant of diphtheria toxin (or tetanus toxoid) have been shown to be highly immunogenic in mice and rabbits. In a phase II immunogenicity and safety clinical trial, conducted by the Medical Research Council in the Gambia, infants were given 1-3 doses of serogroups A and C CPS conjugated to CRM_{197}(Cross Reactive Material 197), or given two doses of plain A and C CPS[4]. The level of antibodies to serogroup A CPS increased steadily following each booster dose, whereas serogroup C CPS antibodies showed lower levels following the two-dose schedule when compared with the single- or triple-dose schedules. It was therefore suggested that serogroup C CPS, given at the age of two months, may induce a state of hyporesponsiveness which can be overcome by adopting a three-dose immunisation schedule[4].

CRM_{197}-conjugated preparations of serogroups A plus C CPS and serogroup C CPS have undergone phase II clinical trials among young British infants in Gloucester and Oxford, respectively[5]. One of the objectives is to assess the safety and immunogenicity of these vaccines when given to infants aged 2, 3 and 4 months with measuring the persistence of antibodies at 12 months. Fairley *et al.* recently reported on the Group A plus C CPS clinical trial and showed that the vaccines are safe and immunogenic, and bactericidal antibodies are detectable at one month after vaccination[6]. However, by 14 months, the antibody level dropped to just above prevaccination levels. The levels of serum bactericidal antibodies required for protection against serogroup C disease is unknown and, in adults, even low levels of antibodies are thought to be protective. However, from the available data, it is not easy to predict protective efficacy of conjugate vaccines among young infants.

Group B CPS vaccines

The CPS of serogroup B meningococci consists of repeated residues of α-(2-8)-linked oligomers of sialic acid, 2-8-α-*N*-acetylneuraminic acid, which serves as an important virulence factor and protective antigen for the organism. *Escherichia coli* K1, a major cause of neonatal meningitis and septicaemia, has an immunochemically identical CPS to that of Group B meningococci and it is an equally poor immunogen. Other organisms which possess the same type of surface antigen include *Pasteurella haemolytica* serotype A-2 and *Moraxella nonliquefaciens*. Candidate vaccines based on the native Group B polysaccharide (B-CPS) induce a transient antibody response of predominantly IgM isotype. Unlike Groups A and C, changing the molecular weight of a Group B vaccine preparation did not induce an immune reponse in human volunteers. It is interesting to note that meningococcal C-CPS, is a homopolymer of 2-9-α-*N*-acetylneuraminic acid and only differs from B-CPS by one linkage.

A number of possible explanations have been given for the poor immunogenicity of the Group B CPS, including sensitivity to neuraminidases and immunotolerance due to its similarity to sialic acid moieties in human tissues. There is, therefore, a concern that forced induction of an antibody reponse to these sialic acid moieties might break the immune-tolerance and cause autoimmune disease and this has tended to discourage attempts to improve the immunogenicity of Group B polysaccharides. Nevertheless, attempts are continuing to produce a CPS-based Group B vaccine. It has been proposed that conformational determinants on the Group B CPS, as presented on the intact organisms, raise antibodies that do not cross-react with the straightforward linear α-(2-8) linked determinants. The conformational structure is therefore seen to be more important in the generation of protective immunity than the primary polysaccharide structure. Various ways of stabilising the molecule in order to present an appropriate conformation, have therefore been investigated. These include the formation of non-covalent complexes of B-CPS with outer membrane proteins, the binding to $Al(OH)_3$ and the conjugation of CPS to carriers such as tetanus toxoid or CRM_{197}[7]. Data following animal and human trials are conflicting, but so far no clear breakthrough has been obtained and it might be some years before the full potential of such a B-CPS-carrier conjugated vaccine is evaluated. Another approach to generate T-cell dependent protective IgG responses has involved attempts to modify the structure of B CPS itself prior to conjugation, by replacing the *N*-acetyl groups of the sialic acid with *N*-propionyl groups[7,8].

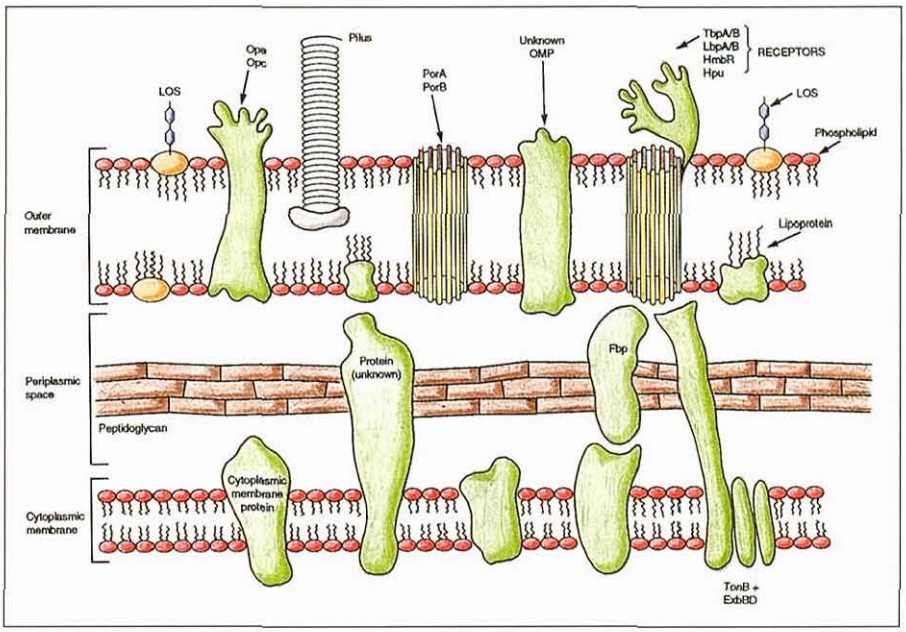


Figure 2. The membrane structure of *N. meningitidis*, showing surface-exposed outer membrane antigens. PorA and PorB= Prines. Opa and Opc = Class 5 Opacity proteins. OMP= Outer membrane protein. LOS= Lipo-oligosaccharide. TonB+ExbBD= Components of Ton-dependent iron-utilisation * = Specific receptors for transferrin (TbpA/B), lactoferrin (LbpA/B), haemoglobin (HmbR), haemoglobin-haptoglobin utilisation (Hpu) and iron (Fbp).

No doubt, considerable pre-clinical safety evaluation will be necessary before such vaccines enter human trials.

Vaccines based on non-capsular antigens

The fact that second episodes of meningococcal disease are rare in the absence of immunodeficiencies, irrespective of the serogroups of the infective organisms, strongly suggests that antibodies against non-capsular antigens contribute to long-lasting and cross-protective immunity. The shortcomings of the currently available CPS vaccines and the poor immunogenicity of Group B-CPS has helped considerably to focus attention on some of the non-capsular antigens in the search for the ideal vaccine (**Figure 2**). Major focus in the past decade has gone to the two main meningococcal porines, PorA (class 1, the serosubtype antigen) and PorB (class 2 or 3 protein, allels of the serotype antigen). A considerable amount of work has been undertaken in examining antibody responses to them, both in humans and in animals. Antibodies against PorA and PorB have been detected in both immunized and infected individuals. Based on the immunological hypervariability of these antigens, meningococcal strains are sub-divided into serotypes and serosubtypes. Within any particular epidemiological setting, the majority of strains causing disease belong to only a limited number of serotypes and serosubtypes. However, the predominant serotypes and serosubtypes associated with disease in any one country change from time to time and with increased international travel, global dissemination of new outbreak-associated strains is common. For example, until recently, serotype 15 and serosubtype P1.7,16 strains were found responsible for the majority of infections in England and Wales; now, serotype 4 and serosubtype P1.4, as well as non-typable/non-subtypable, strains are currently the most frequently isolated in these two countries [9]. These changes will have important implications for the design of vaccines based on serotype and serosubtype antigens.

In any case, if such vaccines were to be successfully developed, it might prove necessary to include several serotype/serosubtype proteins in one vaccine, and to reformulate the vaccine from time to time as the prevalent serotypes and/or serosubtypes changed. Attempts have been made to express selected epitopes of the PorA protein on the surface of *E. coli*[10]. Another approach has been to construct vaccine strains of *N. meningitidis* capable of expressing more than one serosubtype epitope of the PorA protein. Recently, a hexavalent vaccine based on PorA protein from dominant serogroup B isolates from the United Kingdom and the Netherlands has been developed, which also contains some other proteins[11]. In Phase I human trials only half of the volunteers demonstrated a rise in bactericidal antibody levels and a phase II trial is now underway in Gloucester in the UK, the results of which are expected shortly[5].

The class 5 proteins, Opa and Opc, are another group of surface-exposed proteins which have been considered as vaccine antigens. These proteins undergo phase variation and Opa is a hypervariable protein, whereas Opc is not always expressed. These proteins have been implicated in adhesion to epithelial and endothelial cells and possibly in interaction with monocytes and polymorphonuclear cells[12]. The value of antibodies to class 5 proteins in human protection is uncertain, although IgA antibodies on the mucosal surface might possibly be useful.

Iron-regulated proteins

In recent years, iron-regulated proteins have attracted considerable attention as possible vaccine candidates. Under iron restriction, meningococci express several novel proteins which appear to be suppressed under iron-sufficient growth conditions. Many of these iron-regulated proteins are involved in iron acquisition from the host's iron sources. These proteins include highly specific receptors for human transferrin, lactoferrin, heme and haemoglobin, as well as iron-binding proteins and other proteins of uncertain function such as FrpB. The best studied so far, in terms of vaccine candidature, are the two major components of the *transferrin receptor, the transferrin-binding proteins (Tbps)*, TbpA and TbpB[13]. It is interesting that anti-Tbp antibodies can inhibit transferrin binding, iron-acquisition from transferrin and growth of the organism in the presence of transferrin as a sole source of iron. TbpB shows considerable molecular and antigenic heterogeneity amongst different strains of *N. meningitidis*, however, both Tbps have characteristics of safe and broadly cross-reactive vaccine candidates. They are surface-exposed, immunogenic in humans and animals, and antibodies to their native structures are bactericidal to homologous and many heterologous meningococcal strains, including strains from various serogroups, serotypes and serosubtypes[13]. It is anticipated that the Tbps will undergo phase I clinical trials among human volunteers and results should be available in a year or so.

The vaccine potential of other iron-regulated proteins has not yet been assessed and it is feasible that they may share important immunological characteristics with the Tbps. Further progress in this area now requires detailed knowledge of the antigenic structures of these molecules, and their interaction with the host iron-carrying molecules. A meningococcal vaccine based on, or enriched with, iron-regulated proteins from one or more strains in combination

with conventional polysaccharide-based vaccines might increase the spectrum of strains against which protection can be achieved to include serogroup B strains.

Recent trials and future prospects

Several serogroup B meningococcal vaccines based on outer membrane proteins have now been developed and tested in randomized clinical trials. However, the only vaccines to demonstrate significant protective efficacy are those developed in Norway and Cuba[14,15]. These latter two vaccines consist of outer membrane vesicles (OMVs) enriched in PorA and PorB proteins and combined with aluminium hydroxide as adjuvant, the idea of using membrane vesicles being to display the outer membrane proteins in as native a conformation as possible. However, it is important to note that these vaccines do contain significant amounts of other antigens in addition to the porines.

In Norway, a placebo-controlled, randomised double-blind trial was conducted among approximately 170,000 school children aged 14-16 years. This vaccine, based on the Norwegian epidemic meningococcal strain (B:15:P1.7,16), produced a point estimate of protective efficacy of only 57% after a 30-month follow up[14]. The Cuban vaccine, based on a Cuban epidemic strain (B:4:P1.15), also contains serogroup C meningococcal CPS and some other higher molecular weight proteins[15]. In a double-blind, placebo-controlled efficacy trial among 106,252 Cuban children aged 10 to 16 years the vaccine produced a point estimate of protective efficacy of 83% after 16 months' follow-up. Following this trial, mass immunisation of the population between three months and 20 years of age has been carried out in Cuba, with the vaccine now being incorporated into the country's routine paediatric vaccination programme. However, in Sao Paulo, Brazil, the Cuban vaccine was used in a case-control study and vaccine efficacy varied by age. Among children aged four years and older, a point estimate of vaccine efficacy of 74% was obtained, suggesting that the vaccine is effective in older children and adults. Substantially lower estimates of efficacy were obtained for children under four years and no protection at all could be demonstrated in children under two years of age[16].

In order to address some of the unresolved issues, including differences in efficacy between the Cuban and Norwegian vaccines, a multinational collaborative study, sponsored by the World Health Organisation, was undertaken in Iceland in 1992-1993. This prospective, randomised, double-blind study compared the reactogenicity, immunogenicity and serum bactericidal activity elicited in 408 young adults by two or three doses of either the Cuban or Norwegian OMV vaccines. A non-conjugated polysaccharide serogroup A plus C meningococcal vaccine was used as a control. The overall results of the Icelandic study were disappointing[17] and despite extensive studies during and after the Icelandic trial, the issue of the discrepancies between the Cuban and Norwegian vaccine trials has not been resolved. As a consequence, the two vaccines have since undergone another comparative clinical trial in Chile, the results of which may also become available in the near future.

Clearly, there has been considerable activity in the meningococcal vaccine field over the past decade or so. The advances in the development of polysaccharide-conjugate vaccines should eventually lead to more effective vaccines against Group A and C disease, that will protect young infants. However, the problems of a Group B meningococcal vaccine remain. Currently, considerable efforts are being made to evaluate the safety and immunogenicity of polysaccharide-based vaccines in primates[7]. Although preliminary data are promising, more safety evaluation will likely be required before the idea of such vaccines becomes accepted and the vaccines used in humans. Attempts at producing an effective protein-based meningococcal vaccine are also encouraging but again a number of issues need to be resolved.

Unfortunately, no reliable serological correlates of protection exist so far, nor does an universally accepted animal model for estimating protecting potency. The critical test for all vaccine candidates is still protection in humans, and no amount of evaluation in animals can substitute for a good clinical trial. The high degree of adaptation of meningococci to their human hosts, manifested through the continuous antigenic evolution of their surface-expressed macromolecules, also gives cause for concern. But the fact that most adults do not develop meningococcal disease despite presumed continuing exposure to pathogenic meningococci, and that second attacks of the disease in children or adults are very rare, confirm that natural protection is possible and usually highly effective. Thus there are grounds for optimism for the prospects for meningococcal

Continued on page 8 column 2

The Role of the *Bacillus* genus in Infection

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Introduction

Aerobic, Gram-positive, spore-forming (or aerobic spore-bearing – "ASB") rods (**Figure 1**) were traditionally placed within the genus *Bacillus* from the beginnings of bacteriology as a science. However, it was clear from earliest days that ASBs comprised an extreme diversity of species and taxonomists struggled in the 1960s and 1970s to put order and organisation into their classification. Credit goes to Gordon and colleagues[1] for the first truly effective organization of members of the genus based, in the first place, on the shape of the spores, their position in the sporulating vegetative cell (sporangium) and whether or not they produced swelling of the sporangium. The species were then further classified by other physiological (e.g. motility, parasporal bodies) and biochemical characteristics. In the clinical laboratory, this classification and identification system is still the most practical for the principal species encountered in the context of infection.

The heterogeneity within the genus became even more obvious from DNA base composition (mol% G+C (Guanine+Cytosine)) and DNA/DNA reassociation studies carried out in the late 1970s and early 1980s with DNA base compositions found to range at the extremes from 31 mol% G+C (*B. circulans*) to 69% (*B. thermocatenulatus*) and several examples of ranges broader than 5% (sometimes much more than 5%) occurring among isolates placed by classical methods into a single species[2,3]. More recently still, rRNA sequence analysis on type strain cultures of 51 *Bacillus* species led to the conclusion that the current genus should be reconstituted into at least five separate genera[4].

There is no lack of agreement among taxonomists that *Bacillus* should be subdivided into a number of different genera but consensus on how this should be done has been less easy to achieve. Periodic rationalization of the species and reduction in their numbers is invariably followed soon after by the addition again of new names and new species. Currently the genus contains >85 validly published species and many others that await full study (NA Logan, personal communication). Action to regroup a number of the species more appropriately has begun, however, with their recent transfer to four new genera. For example, of the species mentioned in this text or listed in **Table 1**, *B. alvei*, *B. macerans* and *B. polymyxa* have been included with about a dozen other former *Bacillus* species in a new genus *Paenibacillus*[5] and *B. brevis* now belongs to the genus *Brevibacillus*[6].

Table 1. A generalized overview of *Bacillus* infections*

Species	§Degree (1-4+) to which associated with						
	Food poisoning	Bacteraemia/ septicaemia	Wound infection	Severe ophthalmitis	Other human**	Bovine mastitis	Bovine/ovine abortion
B. alvei					1+		
B. anthracis		4+	4+		2+		
B. brevis		1+					
B. cereus	4+	3+	4+	3+	2+	3+	2+
B. circulans		1+	1+		1+		
B. coagulans		2+					
B. licheniformis	2+	2+			1+	3+	3+
B. macerans		1+					
B. pumilus	1+				1+		
B. sphaericus	1+	1+			1+		
B. subtilis	3+	1+			1+		
B. thuringiensis			1+	2+		1+	

* Modified from Turnbull and Kramer[9] and §based on published reports of occurrence.
** Abscess, burns, ear infection, endocarditis, meningitis, peritonitis, pneumonia and other respiratory infections, urinary tract infection.

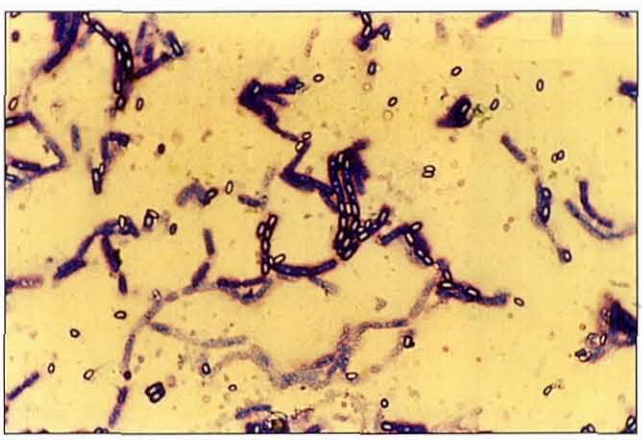

Figure 1. Sporulating vegetative cells and free spores of the New Hampshire strain of *Bacillus anthracis*. In the short chains of vegetative cells, the spores can be seen to lie centrally without swelling the cell (sporangium). Residual capsule (pink) can be seen around many of the cells. (Polychrome methylene blue stained smear from a colony on nutrient agar containing 0.7 % bicarbonate, incubated at 37°C in a candle jar; oil immersion [x100] lens).

Clinical and Public Health Importance

Few members of the genus *Bacillus* are associated with primary infections. However, many others are of clinical importance in a variety of other ways, such as in being producers of antibiotics, or being the basis of antibiotic assays or of tests for validation of sterilization procedures and of disinfectants. The best known of the many peptide or aminoglycoside antibiotics produced by *Bacillus* species are bacitracin from *B. subtilis* or *B. licheniformis*, polymyxin from *B. polymyxa*, and gramicidin from *B. brevis*. *B. cereus*, *B. pumilus* and *B. stearothermophilus* are used in standard antibiotic assays. Spores of *B. stearothermophilus* are the basis of at least one commercial test for heat sterilization procedures. Spores of *B. cereus* are used to validate disinfectant efficacy and those of *B. subtilis* var niger (*B. globigii*) are commonly used for monitoring fumigation. Other strains of *B. subtilis* find use in systems that sterilize using hydrogen peroxide with heat. One unusual application of spores of a strain of *B. cereus* has been as the active ingredient of an anti-

diarrhoeal formulation prescribed in certain countries of Europe. Of ever increasing importance to better understanding of disease and improved diagnosis are the DNA polymerases and restriction endonucleases of a number of *Bacillus* species.

A comprehensive list of commercially important products obtained from *Bacillus* species, including those which fall within the healthcare category, is given by Zukowski[7]. The list includes a large number of enzymes together with a number of antibiotics, vitamins and insecticides and a miscellany of amino acids, carbohydrates and food or feed additives.

The resistance of *Bacillus* spores to heat, radiation and disinfectants makes them troublesome contaminants in surgical theatres, surgical dressings and pharmaceutical products. As normal components of dust, they are ubiquitous in natural, domestic, industrial and hospital environments and they pose a daily problem in the routine diagnostic laboratory where pathogens must be detected and identified from background normal environmental flora. They are, unsurprisingly, the common bacteriological contaminants of the injection paraphernalia of drug addicts[8].

As a whole, therefore, *Bacillus* species are of considerable importance in many aspects of health care.

Bacillus in disease

The only obligate pathogen within the genus is *B. anthracis*, the agent of anthrax. Because of the bad name acquired as a result of its germ warfare associations, a number of popular misconceptions have arisen about this disease. It is primarily a disease of herbivores; humans are incidental hosts, almost invariably acquiring it from contact with the carcasses of animals that have died of the disease or with products from such animals, in particular, hair, wool, hides and bones. Circumstantial evidence indicates that humans are fairly resistant to infection. The putative aggressive use of anthrax spores is based on ensuring exposure of humans and animals to overwhelmingly massive doses, millions of times greater than can be encountered under the worst natural conditions. Anthrax is not, in fact, highly infectious; point source infection rather than cross-infection is almost invariably the rule. It remains an important disease in several countries of Africa, Asia and central and southern Europe while many industrial countries, including Britain, continue to record low numbers of cases in livestock each year with occasional human cases. Sometimes the events in a country like Britain can be traced to contaminated animal products from endemic countries.

The pathogenesis of anthrax and its clinical development are shown in **Figures 2** and **3** respectively. Two virulence factors, the capsule which protects the organism from host phagocytosis, and the toxin with several functions, play important roles. The root of control in endemic areas is vaccination of the livestock; in non-endemic areas suffering sporadic cases or outbreaks, antibiotic treatment of members of affected herds showing suspect symptoms may be part of the control measures (not permitted in livestock management in some countries, however) and carcasses are incinerated followed by disinfection and decontamination of affected premises. Vaccination may again be considered advisable.

Despite being a disease known from antiquity, there remains a great deal not understood about anthrax. For example, significant strain differences have never been clearly demonstrated; those that do exist are unquantifiable. That is to say, regardless of their isolation histories – what animal, human or environmental specimens they came from, or what country – there is no readily available way of distinguishing one isolate from another apart from poorly definable differences such as colonial morphology or degree of capsule formation. However, it is sometimes hard to explain different epidemiology patterns except in terms of strain differences. For example, it is common experience that only one animal species is affected in an outbreak while other susceptible animals, apparently equally exposed, remain unaffected. Development of a strain differentiation system for *B. anthracis* is the subject of current research.

The involvement of other *Bacillus* species in disease is summarised in **Table 1**. It is seen that several species have been associated with a wide range of infection types but it is probable that the majority of cases represent underlying immunological, metabolic or other disorder, trauma (surgical, severe burns, etc.) or drug abuse on the part of the patient. Other than *B. anthracis*, only *B. cereus* and its close relative, *B. thuringiensis*, have clearly identifiable virulence factors by which to explain any pathogenic mode of action, although all *Bacillus* species produce a wide variety of extracellular enzymes, some of which may play a direct or indirect role in the syndrome with which the bacteria have been associated. By far the greatest number of reports of non-anthrax *Bacillus* infections concern *B. cereus*.

Reports of *Bacillus* food poisoning have been lower in the 1990s than in the previous two decades. This possibly reflects, to some extent at least, improved education of food handlers and the public in general in food hygiene. These types of food poisoning are generally regarded as inconvenient and self-limiting, requiring only symptomatic treatment. They are not normally regarded as life-threatening to any particular population group but there are at least four published reports of fatal cases of *B. cereus* food poisoning[10]. Recent reports of food poisoning attributed to

Figure 2. Terminal haemorrhagic exudation from the nose (A - springbok), mouth (B - blue wildebeest), and/or anus (C - elephant) at death is a characteristic sign of anthrax. Falling on the ground, the vegetative *B. anthracis* in the blood sporulate on exposure to oxygen in the air where they await uptake by the next host grazing over the site any time from a day or so to several decades later. The new host takes in the spore, usually by ingestion. Soil or spiky grass, or other items capable of causing abrasions in the alimentary canal, make a lesion where the spores can lodge and be carried to the lymphatics and spleen where they multiply during the incubation period of a few days. The anthrax toxin is formed during multiplication and ultimately results in the breakdown of the organs in a sudden burst releasing the toxin and vast numbers of bacilli into the blood stream. This is reflected in the rapid onset of symptoms with rapid descent from clinically normal to death in a few hours. The toxin also causes generalised breakdown of blood vessel walls leading to the terminal haemorrhage – and the cycle of infection is complete. (Photographs taken in the Etosha National Park, Namibia).

Table 2. *Bacillus* food poisoning, England & Wales 1990-95*.

B. cereus			*B. subtilis*			*B. licheniformis*			Mixed *Bacillus*§			All cases
G	F	S	G	F	S	G	F	S	G	F	S	
48	15	30	13	4	15	2	2	4	9	1	7	644

G, general outbreaks; F, family outbreaks; S, sporadic cases

§ Species involved (in various combinations): *B. cereus / subtilis / licheniformis / sphaericus / pumilus / amyloliquefaciens*

*Data kindly supplied by the PHLS Communicable Disease Surveillance Centre and Food Hygiene Laboratory, Central Public Health Laboratory, Colindale.

Bacillus species in England and Wales are given in **Table 2**.

B. cereus, in particular, has long been associated with two distinct types of foodborne intoxications and with a particularly wide spectrum of other types of clinical conditions[11] (see **Figure 4**). More recently, *B. subtilis* has also come to feature in food poisoning statistics too frequently to dismiss as unimportant.

The two *B. cereus* food poisoning syndromes are characterised either by nausea and vomiting 1 to 5 hours (emetic type) or by diarrhoea and abdominal pain 8 to 16 hours (diarrhoeal type) after ingestion of the contaminated food. These are attributed to distinct emetic and diarrhoeal enterotoxins produced under different circumstances of faulty food preparation. A wide range of foods can be involved in the diarrhoeal-type syndrome; under improper storage conditions, contaminating *B. cereus* will multiply, at the same time producing the responsible toxin. In contrast, some 90 % of incidents of the emetic type of food poisoning are associated with rice dishes[13].

A particularly nasty haemolysin has now been incriminated as being responsible for both the diarrhoeal syndrome and the periodic severe wound, eye, systemic or other *B. cereus* infection[14]. The emetic toxin has been identified recently by Japanese workers as a small molecular weight dodecadepsipeptide[15] which they have named 'cereulide'.

B. subtilis-associated food poisoning somewhat resembles the emetic syndrome of *B. cereus* with vomiting as the predominant symptom although diarrhoea is relatively common and the association with rice dishes is less pronounced. *B. licheniformis* food poisoning, on the other hand resembles more the diarrhoeal type of *B. cereus* food poisoning. However, no *B. subtilis* or *B. licheniformis* toxins analagous to those of *B. cereus* have been described. Likewise, frequent associations of *B. licheniformis* with bovine and ovine abortion[16] and with serious ascending vaginal infections in cows which lie on maize sileage[17] suggest a pathogenic mechanism which has yet to be elucidated. This is certainly an area worthy of research.

Now and then, statements alleging production of toxins by *Bacillus* species other than *B. cereus* are encountered[18], but the nature of these toxins and their relevance to infection have yet to be established. That these species are generally regarded as safe by the US Food and Drug Administration remains important to their use, or proposed use, in production of materials for consumption or clinical purposes[19], and a level-headed response to reports of their associations with infection is necessary. On the other hand, many *Bacillus* infections probably go unrecognized because the organisms are all too readily dismissed as contaminants and clinical

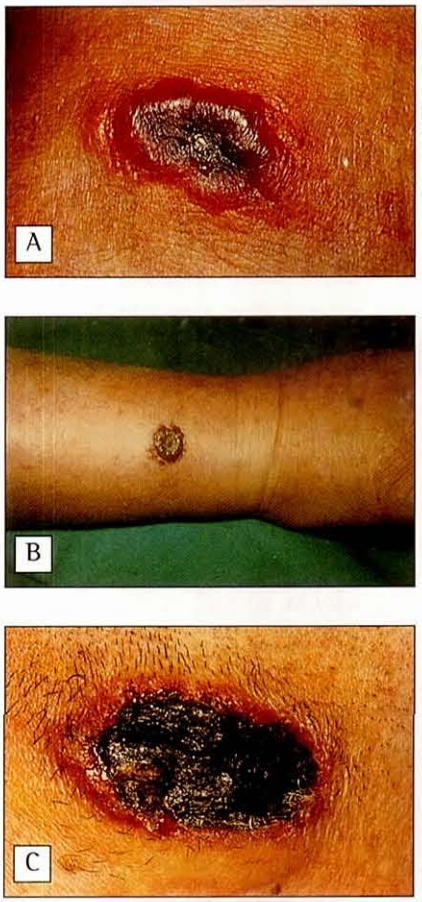

Figure 3. The developing cutaneous lesion of anthrax. The initial lesion is a small pimple 1-3 days after infection. Over the next 24h, this becomes encircled by a ring of vesicles and the central pimple ulcerates and dries to form the characteristic black "eschar". Resolution begins at about 10 days (regardless of treatment). (A) 4 days, (B) 7 days and (C) 11 days after first seen. Note, in B, the extensive oedema, also a typical characteristic of cutaneous anthrax. If it occurs around the neck in association with a lesion on the face or neck, death can result from asphyxiation if tracheotomy is not performed in time. Despite its appearance, the lesion is not painful, unless secondarily infected. (Photographs kindly supplied by Dr R.M. Pfisterer, Grüt, Switzerland, and reproduced from Schweiz med Wschr 1991; 121: 813-25, by kind permission)

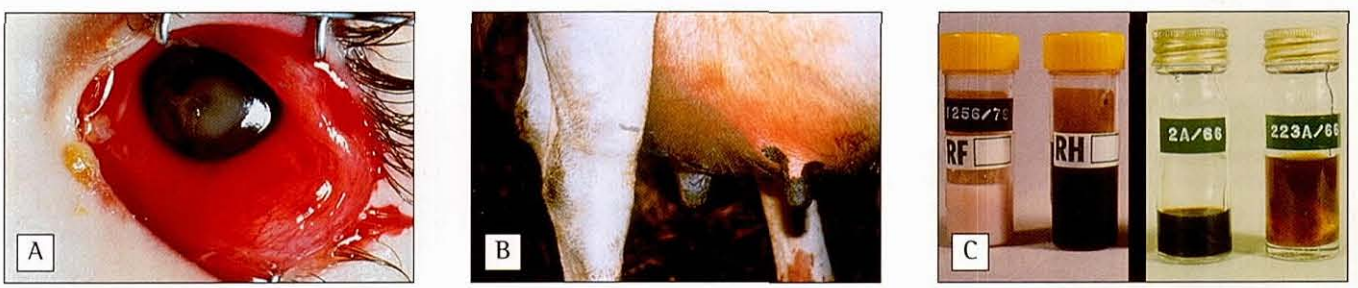

Figure 4. Severe *Bacillus cereus* infections. (A) Panophthalmitis following a penetrating eye injury. (Kindly supplied by D.M. O'Day, MD, Department of Ophthalmology, School of Medicine, Vanderbilt University, Tennessee. (B) Blistered left teat and discoloured right teat due to gangrenous bovine mastitis; *B. cereus* can be the causative agent of this type of infection. (Kindly supplied by R.W. Blowey, FRCVS, Wood Veterinary Group, Gloucester. (C) Milk from cows with *B. cereus* mastitis. In the early stages, the milk is tinged pink with blood; in the fulminant infection, the milk is replaced by serosanguinous exudate. (Kindly supplied by the late K.G. Johnston, MACVSc, Department of Veterinary Clinical Studies, University of Sydney). [Reproduced from Parry *et al*[12], by kind permission of Mosby-Wolfe, London].

laboratories should be strongly encouraged to interpret isolations of *Bacillus* species very critically when they occur.

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References

1. Gordon, R.E., Haynes, W.C. and Pang, G.H-N. (1973). *The Genus* Bacillus. USDA, ARS, Agricultural Handbook, No. 427. U.S. Government Printing Office, Washington, D.C.
2. Priest, F.G. (1981). In: *The Aerobic Endospore-forming bacteria*. Berkeley, R.C.W. and Goodfellow, M. (eds). Academic Press, London, pp 33–57.
3. Logan, N.A. (1994). *Bacterial Systematics*. Blackwell Scientific Publications, Oxford, p 168.
4. Ash, C.A., Farrow, J.A.E., Wallbanks S. and Collins, M.D. (1991). *Lett. Appl. Microbiol.* **13:** 202–206.
5. Heyndrickx, M., Vandemeulebroecke, K., Scheldeman, *et al.* (1996). *Int. J. Syst. Bacteriol.* **46:** 988–1003.
6. Shida, O., Takagi, H., Kadowaki, K. and Komagata, K. (1996). *Int. J. Syst. Bacteriol.* **46:** 939–946.
7. Zukowski, M.M. (1992). In: *Biology of Bacilli. Applications to Industry*. Doi, R.H. and McGloughlin, M. (eds). Butterworth-Heinemann, Boston, pp 311-337.
8. Tuazon, C.U., Murray, H.W., Levy, C., *et al.* (1974). *J. Am. Med. Assoc.* **241:** 1137–1140.
9. Turnbull, P.C.B. and Kramer, J.M. In: *Manual of Clinical Microbiology*. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (eds). 6th ed. ASM Press, Washington, pp. 349–356.
10. Mahler, H, Pasi, A., Kramer J.M., *et al*, *New Eng. J. Med.* **336:** 1142–1148.
11. Drobniewski, F.A. (1993). Clin. Microbiol. Rev. **6:** 324–338.
12. Parry, J.M., Turnbull, P.C.B. and Gibson, J.R. (1983). *A Colour Atlas of Bacillus Species*. Wolfe Medical Atlases – 19. Wolfe Medical Publications Ltd., London, 272pp.
13. Kramer, J.M. and Gilbert, R.J. (1992). In: *Food poisoning*. Tu, A.T. (ed). Handbook of Natural Toxins, Vol 7. Marcel Dekker, Inc., New York, pp 119-153.
14. Beecher, D.J. and MacMillan, J.D. *Infect. Immun.* **58:** 2220–2227.
15. Shinagawa, K., Konuma, H, Sekita, H. and Sugii, S. (1995). *FEMS Microbiol. Lett.* **130:** 87–90.
16. Logan, N.A. (1988). *J. Med. Microbiol.*, **25:** 157–165.
17. Blowey, R. and Edmondson P. (1995). *Mastitis Control in Dairy Herds. An Illustrated and Practical Guide*. Farming Press Books, Ipswich, p.41.
18. Beattie, S.H. and Williams, A.G. (1996). *J. Appl. Bacteriol.* **81S:** xxiv.
19. Priest, F.G. (1989). In: *Bacillus*. Harwood, C.R. (ed). Plenum Press, London, pp. 293–320.

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vaccines. Nevertheless, it may be another decade or so before safety and efficacy data from large scale clinical trials become available. It may be even longer before a broadly or universally cross-protective vaccine becomes available for incorporation into childhood immunisation programmes.

There is clearly much more to learn about the human immune response to different meningococcal antigens if we are to develop rationally a highly effective broadly cross-protective vaccine. The protein-based vaccines that have undergone clinical trial to date are very complex preparations and it is not clear which of the several components really contribute to protection in man. Detailed characterisation of vaccines undergoing clinical trials is crucial if we are to interpret trial results sensibly and be in a position to make vaccines of consistent composition, and efficacy. Efforts to understand the mechanisms by which vaccines confer protection should be intensified and proper tests for evaluating possible immunological correlates of protection explored. More information is needed on the B-cell and T-cell responses to meningococcal antigens, and on the role of cellular immunity in protection against this infection.

References

1. Vieusseaux M. (1805) *J Med Chir Pharm*, **II:** 163–165.
2. Weichselbaum. (1887) *Fortsch Med*, **5:** 573–575.
3. Halstensen A., *et al.* (1989) *Scand J Infect Dis*, **21:** 267–276.
4. Twumasi P.A.J., *et al.* (1995) *J Infect Dis*, **171:** 632–638.
5. Herbert M.A., Heath P.T., Mayon-White R.T. (1995) *Com Dis Rep*, **5:** R130–135.
6. Fairley C.K., *et al.* (1996) *J Infect Dis*, **174:** 1360–1363.
7. Delvi S.J.N., *et al.* (1997) *Infect Immun*, **65:** 1045–1052.
8. Jennings H.J., Gamian A., Ashton F.E. (1987) *J Exp Med*, **165:** 1207–1211.
9. Jones D.M., Kaczmarski E.B. (1995) *Com Dis Rep*, **5:** R125–R130.
10. McCarvil J., *et al.* (1993) *Mol Microbiol*, **10:** 203–213.
11. Peeters C.C.A.M., *et al.* (1996) *Vaccine*, **14:** 1009–1015.
12. McNeil G., Virji M. (1997) *Microb Pathog*, **22:** 295–304.
13. Ala'Aldeen D.A.A. (1996) *J Med Microbiol*, **44:** 237–243.
14. Bjune G., *et al.* (1991) *Lancet*, **338:** 1093–1096.
15. Sierra G.V.G., *et al.* (1991) *NIPH Ann*, **14:** 195–210.
16. De Moraes J.C., *et al.* (1992) *Lancet*, **340:** 1074–1078.
17. Perkins B.A., *et al.* (1994) In: *Neisseria 94*. Evans J.S., *et al.* (Ed). NIBSC, Potters Bar, UK. 438.