PERSPECTIVES: MICROBIOLOGY

A Furtive Pathogen Revealed

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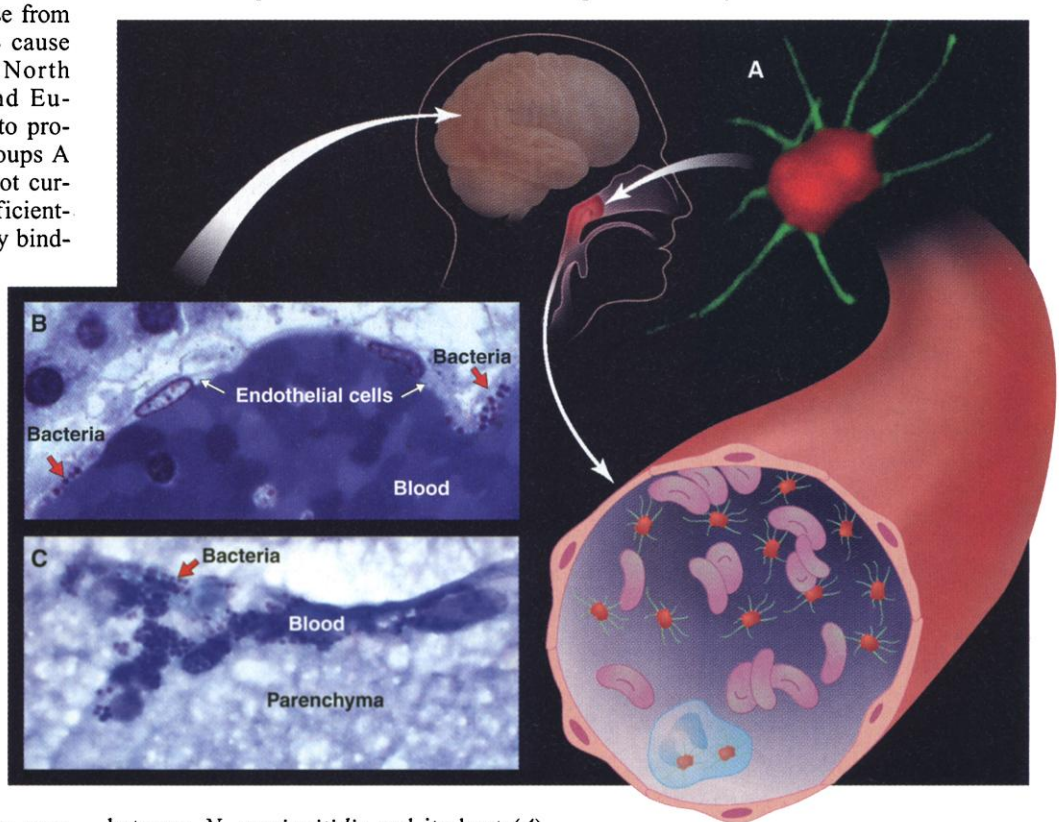
The bacterial pathogen *Neisseria meningitidis* is the cause of septicemia and meningococcal meningitis—a life-threatening inflammation of the meninges (the membranes that surround the brain and spinal cord). There are five serogroups (A, B, C, Y, and W135) that cause disease. Bacteria from serogroup A are the cause of meningitis epidemics in sub-Saharan Africa, whereas those from serogroup B cause disease in North America and Europe. Although there is a vaccine to protect against infection with serogroups A and C, a serogroup B vaccine is not currently available. *N. meningitidis* efficiently crosses the blood-brain barrier by binding directly to brain capillaries of the choroid plexus and parenchyma. The virulence factors that enable *N. meningitidis* to readily cross this tight barrier may be valuable antigens for inclusion in a vaccine. On page 1809 of this issue, Tettelin and colleagues provide the complete genome sequence of *N. meningitidis* strain MC58 (serogroup B) (*1*). They compare its sequence with that of *N. meningitidis* strain Z2491 (serogroup A) (*2*) and of *Haemophilus influenzae*, another pathogen that causes meningitis. In an accompanying paper on page 1816, Pizza and co-workers (*3*) report the results of mining the complete genome sequence of strain MC58. They identified seven new, highly conserved proteins exposed on the surface of the *N. meningitidis* bacterium that hold promise as candidate vaccine antigens.

Enhanced online at www.sciencemag.org/cgi/content/full/287/5459/1767

Paradoxically, *N. meningitidis* is found only in humans, suggesting that its ability to cause disease is likely to be an accidental side effect of its life cycle. This pathogen colonizes the nasopharynx of a substantial proportion of the population without causing any symptoms. For unknown reasons, in a small percentage of asymptomatic carriers the bacteria enter the bloodstream, cross the blood-brain barrier, and invade the meninges of the brain. This is followed by local production of cytokines and an inflammatory response. Various bacterial components—such as the carbohydrate coat that encapsulates *N. meningitidis*, type IV pili (the long, threadlike projections on the bacterial surface), outer membrane (opacity) proteins, and proteins that chelate iron—have been implicated in the interaction between *N. meningitidis* and its host (*4*) (see the figure).

The best-defined virulence factor of *N. meningitidis* is its polysaccharide coat. This coat enables the pathogen to enter the bloodstream because nonencapsulated strains are found only in the nasopharynx of asymptomatic carriers and not in the blood. The three major serogroups (A, B, and C) are defined according to the chemical composition of their polysaccharide coats. Type IV pili are essential for the interaction of meningococcal bacteria with host cells, and have been implicated in colonization of the nasopharynx and the ability of the pathogens to cross the blood-brain barrier. They are thought to behave like bacterial sensory organs, initiating interactions between bacteria and host cells (*5*). It remains unclear whether it is the opening of tight junctions between cells of the blood-brain barrier or the transport of bacteria across these cells that enables *N. meningitidis* to enter the brain.

The meningococcal virulence factors characterized so far are also found in other extracellular bacterial pathogens that do not cross the blood-brain barrier. This suggests that *N. meningitidis* has other as yet unidentified virulence factors that account for this capability. In their study, Pizza *et al.* (*3*) examine 350 proteins that are expressed in the bacterial outer membrane. Of these, the investigators identify seven that are conserved



The creature that lurks within. The bacterial pathogen *N. meningitidis* spreads from person to person by direct contact. The pathogen colonizes the epithelia of the nasopharynx where it does not cause symptoms. However, for unknown reasons, in a small percentage of infected asymptomatic individuals the bacteria enter the bloodstream, cross the blood-brain barrier, and invade the brain meninges. (**A**) There are several well-characterized virulence factors of *N. meningitidis* (red) including type IV pili (green). (**B**) Postmortem section of a capillary in the choroid plexus of the brain showing *N. meningitidis* adhering to endothelial cells. (**C**) Postmortem section of brain parenchyma showing *N. meningitidis* adhering to brain capillaries.

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among a number of meningococcal strains and that induce the production of bactericidal antibodies when injected into mice. The fact that these seven proteins are both highly conserved and exposed in the bacterial outer membrane makes them good candidate virulence factors.

It is likely that conserved regions of the meningococcal genome encode essential components of the pathogenesis pathway. Tettelin *et al.* report that 91% of the genome sequence of strain MC58 (serogroup B) is present in the genome of strain Z2491 (serogroup A). The 9% of the genome that differs between serogroups A and B may account for the different ways in which these two serogroups spread through human populations.

The *Neisseria* species include *Neisseria gonorrhoeae* (which colonizes and invades the epithelium of the urogenital tract, where it causes an inflammatory response) and *Neisseria lactamica* (which is not pathogenic). Subtractive hybridization has enabled the construction of a library of genes that are present in one species but not the other (*6*). In addition, comparison of the sequence of *N. meningitidis* with the partial sequence of *N. gonorrhoeae* (*7*) now allows the identification of chromosomal regions that are either specific for *N. meningitidis* or shared with *N. gonorrhoeae*. Those sequences specific to *N. meningitidis* are more likely to be involved in specific aspects of meningococcal pathogenesis, such as septicemia and invasion of the brain meninges. Genomic regions shared by *N. meningitidis* and *N. gonorrhoeae*, but absent in *N. lactamica*, are likely to be responsible for common aspects of the life cycle of pathogenic *Neisseria*, such as colonization of epithelia at the point of entry.

The success of the conjugate vaccine for protecting against *H. influenzae* serogroup B infection demonstrates that efficient protection against bacterial pathogens that cross the blood-brain barrier is possible. The goal of a meningococcal vaccine is not to prevent infection but to block bloodstream dissemination of the pathogen in asymptomatic carriers. Administration of a vaccine antigen such as purified coat polysaccharide early in life could result in the production of bactericidal antibodies that prevent bacteria from entering the bloodstream. Unfortunately, the serogroup B coat polysaccharide is structurally identical to a carbohydrate component of the N-cellular adhesion molecule expressed on the surface of brain cells and so it cannot be used in a vaccine. However, coat polysaccharides of serogroups A and C induce protective antibodies in children older than 2 years of age, and a new conjugate vaccine containing these polysaccharides is now being administered to infants.

Pizza *et al.* (*3*) systematically screened the *N. meningitidis* genome for surface proteins that are highly conserved. They identified 350 candidate vaccine antigens, expressed the genes in *Escherichia coli*, purified the proteins, and immunized mice with them. Of these they identified seven surface proteins that are highly conserved among 22 serogroup B pathogenic strains and are also highly conserved in serogroup A and C pathogens. All seven proteins, which include four lipoproteins and two outer membrane proteins, were accessible to antibodies and evoked efficient antibacterial antibody responses, suggesting that they hold promise as vaccine antigens.

Although the path that leads from finding a vaccine candidate to producing a working vaccine is seldom smooth, the reports by Tettelin, Pizza, and their colleagues stress the enormous potential of bacterial genomics for discovering new therapeutic strategies to fight infectious disease.

References

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PERSPECTIVES: PLANETARY SCIENCE

Glass Beads Tell a Tale of Lunar Bombardment

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By a little over 3 billion years ago, the heavy battering that the early moon and Earth had been receiving from projectiles had diminished to a level comparable with that of today (*1*). Has the bombardment been essentially constant over these past few billion years, or has it increased or declined? Have there been periodic or episodic variations? The answers to these questions contain clues to the source of bodies—asteroids or comets or both—that enter the inner solar system, the influence of impacts on Earth, the current hazard risk, and the dating of surfaces of other inner solar system planets. On page 1785 of this issue, Culler *et al.* (*2*) use radiogenic isotope analyses of lunar samples to persuasively argue that there has been a substantial increase in impacting in the Earth-moon system over the past few hundred million years, following a decline over the previous 3 billion years.

The present impact rate can be estimated from an inventory of Earth-crossing asteroids and comets (*3*). The average rate over the past 3 billion years can be determined by counting the lunar craters that have formed on the 3.1- and 3.3-billion-year-old lava flows sampled by the Apollo 12 and Apollo 15 missions, respectively (*1*). This average lunar rate can be compared with that estimated from Earth's own ~150 impact craters, with appropriate consideration of Earth's greater cross section for attracting projectiles and the greater impact velocities and different crater modification because of its higher gravity.

Earth's craters have the potential for reasonably accurate and precise absolute and stratigraphic ages. Estimates of both the present rate (based on observed Earth crossers) and that over the past 200 or so million years (based on impact structures on older continents and the younger Mississippi lowlands) suggest a rate twice that of the lunar average over the past 3 billion years (*4*). The anomalously high frequency of very large "young" craters on Earth (such as Chicxulub, 65 million years old) is consistent with such a higher rate. However, the uncertainties are high enough to allow the possibility of a fairly constant rate (*1*).

Erosion, sedimentation, and plate tectonics have eliminated much of the older record on Earth, and in the case of smaller craters, even relatively young ones are depleted. It is thus not likely that we can ever substantially improve our database from the terrestrial record. In contrast, the lunar record is superbly retained, but we have limited access to it. In particular, we do

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