CONTENTS

Contributors ix
Foreword Robert Austrian xv
Preface xix

THE BACTERIA 1

GENOME
1. What Is a Pneumococcus?
   Christopher G. Dowson 3

2. Comparative Genomics of Streptococcus pneumoniae: Intrastrain Diversity and Genome Plasticity
   Hervé Tettelin and Susan K. Hollingshead 15

SURFACE ANATOMY
3. Capsules
   Janet Yother 30

4. Choline-Binding Proteins
   Edwin Swiatlo, Larry S. McDaniel, and David E. Briles 49

5. Pneumolysin and Other Virulence Proteins
   Tim J. Mitchell 61

MICROBIAL PHYSIOLOGY
6. Cell Wall Hydrolases
   Rubens López, Ernesto García, Pedro García, and José Luis García 75

7. Transformation
   Sanford A. Lacks 89
CONTENTS
HOST-MICROBE INTERACTIONS  117

EXPOSURE AND COLONIZATION

8. Evolutionary and Population Biology of Streptococcus pneumoniae
   Brian G. Spratt, William P. Hanage, and Angela B. Brueggemann
   119

9. Pneumococcal Carriage
   Derrick W. Crook, Angela B. Brueggemann, Karen L. Sleeman, and
   Timothy E. A. Peto
   136

10. Epidemiology of Pneumococcal Disease
    Jay C. Butler
    148

11. Mechanisms of Carriage
    Jeffrey N. Weiser
    169

INVASION AND INFECTION

12. Inflammation and Host Defense
    Paul Anthony Majcherczyk and Philippe Moreillon
    183

13. Interactions of Streptococcus pneumoniae with the Proteins of the
    Complement Pathways
    Margaret K. Hostetter
    201

14. A Pathogenetic Categorization of Clinical Syndromes Caused by
    Streptococcus pneumoniae
    Daniel M. Musher
    211

15. Attachment and Invasion of the Respiratory Tract
    Elaine I. Tuomanen
    221

16. Pathogenesis of Pneumococcal Meningitis
    Joerg R. Weber
    238

17. Immunodeficiency and Invasive Pneumococcal Disease
    Edward N. Janoff and Jeffrey B. Rubins
    252

TREATMENT AND PREVENTION  281

18. Changing the Ecology of Pneumococci with Antibiotics and Vaccines
    Ron Dagan and Marc Lipsitch
    283
19. Pneumococcal Infections: Therapeutic Strategies and Pitfalls  
   Kathryn M. Edwards  
   314

20. Clinical Relevance of Antibiotic Resistance in Pneumococcal Infections  
   Keith P. Klugman  
   331

   Carina Bergmann, Fang Chi, Shwan Rachid, and Regine Hakenbeck  
   339

22. Macrolide, Quinolone, and Other Non-β-Lactam Antibiotic Resistance in Streptococcus pneumoniae  
   Karita Ambrose and David S. Stephens  
   350

23. The Humoral Immune Response to Streptococcus pneumoniae  
   Clifford M. Snapper, Jesus Colino, Abdul Q. Khan, and Zheng Qi Wu  
   367

24. New Pneumococcal Vaccines: Basic Science Developments  
   James C. Paton  
   382

25. Vaccine-Induced Immunity to Pneumococcal Infection  
   P. Helena Mäkelä and Helena Kayhty  
   403

Index 421
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The pneumococcus, *Streptococcus pneumoniae*, a normal component of the microflora of the human upper respiratory tract and an opportunist pathogen, has been an object of scrutiny for almost a century and a quarter. Its initial isolations have been described succinctly by Welch (93):

The micrococcus lanceolatus was discovered by Sternberg in September, 1880, by inoculation of rabbits with his own saliva. It was next found by Pasteur in December, 1880, by inoculation of rabbits with the saliva of a child dead of hydrophobia. Pasteur's observations were the first to be made public, being announced at a meeting of the Académie de Médecine in Paris on January 18, 1881... Sternberg's first publication on this subject appeared on April 30, 1881.

Both scientists were able to grow the organism in vitro, and their descriptions of the morphology of their isolates were similar (76, 82), each recognizing what are now designated its diplococcal form and its capsule, attributes visible in the first microphotograph of the pneumococcus made by Sternberg (83). Sternberg was able also to recover pneumococci from the saliva of several carriers.

Since the initial observations of Sternberg and of Pasteur, much has been learned about the ecology of the pneumococcus in health and in disease. Studies of the pneumococcal carrier state have revealed that colonization of the human upper respiratory tract may occur on the day of birth (36) and that children may be colonized simultaneously with as many as 4 different capsular types (37) and, over a period of several years and at different times, with 1 or more of 12 different capsular types (55). Carriage of a single type over a period of 3 years in an adult in the absence of illness has also been observed (6, 91). Noteworthy are the findings demonstrating convincingly in an epidemiological study of the pneumococcal carrier state that half those colonized would not have been identified had mice not been inoculated with their upper respiratory secretions (47).

The mammalian lower respiratory tract, in contrast, is highly resistant to pneumococcal infection. Normal mice exposed to an aerosol containing pneumococci suspended in particles small enough to reach the pulmonary
alveoli clear the organism rapidly. Mice infected with influenza A virus do not develop pneumococcal pneumonia when similarly exposed at a time when the viral titer is at its height but in the absence of histologic changes in the lung. Only when there is visible evidence of pulmonary injury or edema does pneumococcal infection develop following such exposure (39). These findings are emblematic of events that, in many instances, find their counterparts in humans. It is highly likely also that viral infection of the upper respiratory tract plays a similar antecedent role in pneumococcal infections of the middle ear.

Although it is probable that pneumococci were visualized in pulmonary secretions by Klebs as early as 1875 and in histologic sections of the lung described by Carl Friedländer in 1882, recognition of the pneumococcus as the principal cause of human lobar pneumonia was not to be clarified for another four years, when Anton Weichselbaum resolved the bitter dispute between Friedländer and Albert Fraenkel as to its etiology (4). It is of historic interest that Christian Gram, working in Friedländer’s laboratory, developed the stain that now bears his name not to distinguish among bacteria but rather to facilitate their visualization in histologic sections of the lung (34).

The 1880s saw rapid extension of the association of the pneumococcus with extrapulmonary foci of infection that are now well recognized. Pneumococci were isolated from blood, from the lung by direct puncture during life, from pleural, cerebrospinal, and joint fluid, and from the middle ear (5). In the same decade, Netter described pneumococcal endocarditis and produced the disease experimentally by damaging a rabbit’s aortic valve and injecting the animal subsequently with pneumococci (67).

Knowledge of the host’s defenses against pneumococcal infection evolved slowly over the final 15 years of the 19th century. In 1885, Fraenkel recorded that a rabbit which recovered from a pneumococcal infection of the ear was refractory to reinfection with the same pneumococcal strain (31). Gamaléia, in 1888, was perhaps the first to suggest the role of phagocytic cells in defense against pneumococcal infection of the lung (33). In 1891, Metchnikoff described agglutination of pneumococci by immune serum (62), and two years later, Issaef reported that immune serum, although lacking both antitoxic and antibacterial properties, promoted phagocytosis (49). In the early 1890s, the Klemperers observed that the offspring of immunized rabbits were immune to infection with the same pneumococcal strain used to immunize the mother, that the serum of an immunized rabbit would protect an unimmunized rabbit against challenge with the same organism, and that the serum of a patient after crisis could protect a rabbit against infection. They also injected several patients subcutaneously with small amounts of rabbit serum (50). The rational development of serotherapy, however, had to await the clear definition of pneumococcal types, intimations of which are discernible in the observations of Bezançon and Griffin (14). In the same period, loss of virulence on passage of pneumococci in vitro and its recrudescence on subsequent passage in vivo were also described (53).

To Fred Neufeld must be credited major contributions to knowledge of the pneumococcus. In 1900, he described the bile solubility of pneumococcus, an observation which greatly facilitated its identification (68); in 1902, he described the Quellung reaction (69); and in 1904, with Rimpau, he showed
that antipneumococcal antibody combines with pneumococci and not with the polymorphonuclear leukocytes that phagocytize them (73). Of equal, if not greater, importance was his definition with Händel of pneumococcal capsular types 1 and 2, making possible the introduction of rational serotherapy of pneumococcal infection (71). Interestingly, neither Neufeld nor others concerned with pneumococcal infection were to use the Quellung reaction in their studies for a quarter century following its original description, the identification of the first 30 pneumococcal capsular types having been achieved with other techniques (22). The term “Quellung” is probably a misnomer; for, as shown by Schiemann and Casper, a combination of a solution of capsular polysaccharide with homologous antibody results in the formation of a liquid gel which differs in no way in its refractile properties from the pneumococcal capsule when exposed to homologous antibody, giving rise to what might be termed more appropriately a capsular precipitin reaction (79). The Quellung technique was introduced by Neufeld and Etinger-Tulczynska as the preferred method for typing pneumococci in 1932 (70).

Both the capsule and the mode of cellular separation after division influence the morphological patterns of the pneumococcus in liquid and on solid media and have given rise historically to several descriptive terminologies at both the microscopic and macroscopic levels (2). The patterns of pneumococcal growth appear to be determined largely by its environment, the organism adapting to conditions optimizing its nutrition and its defense against phagocytosis. In liquid media, pneumococci, whether capsulated or noncapsulated, grow preferentially as diplococci distributed throughout the medium. Continued cultivation of capsulated strains in liquid medium in the presence or absence of antibody is associated with loss of capsulation, no longer essential to survival. Cultivated on solid media for prolonged periods as dispersed colonies, pneumococci, capsulated or noncapsulated, give rise to variants growing in long chains, with pseudomotility providing nutritional advantage over diplococcal forms. By contrast, in liquid media, the filamentous variants are at a nutritional disadvantage, being autoagglutinable, and revert to the diplococcal form. An additional type of morphological variation has been described recently by Weiser et al., again related to environmental circumstances and reflected in the transparence or lack thereof of colonies on transparent solid media (92). Pneumococci isolated from the nasopharynx show a higher ratio of cell wall polysaccharide to capsular polysaccharide and form transparent colonies, in contrast to similar organisms isolated from blood in which the augmented amount of the capsular polymer is associated with an opaque colonial form.

The virulence of a given pneumococcal type, of which 90 have been defined to date (46), is determined both by the chemical composition of the capsular polymer and the amount synthesized (52). Of the two, the former is the more important. In addition to capsular polysaccharides, the pneumococcus produces an additional polymer in this category, the so-called C or cell wall polysaccharide composed in part of a teichoic acid. Discovered during an investigation of the cellular constituents of the pneumococcal cell by Tillett and Francis in 1931 (87), this carbohydrate is common to pneumococci of all capsular types. Noncapsulated pneumococci grown in a suitable environment can give rise to mutants producing a capsule of C-like polysaccharide, the
genes for which occupy a locus separate from those determining the synthesis of conventional capsules (16). Pneumococcal C polysaccharide is of additional interest for its ability to combine with an acute-phase protein of human hepatic origin. Unlike antibody, C-reactive protein appears at the height of the inflammatory response, be it of pneumococcal or unrelated origin, and disappears from the circulation with resolution of the inflammatory process (1).

The pneumococcus has played a major role in the development of immunology, and some of its beginnings in the 19th century have been cited above. Prior to the second decade of the 20th century, it was widely believed that all antigens were proteins. In 1917, Dochez and Avery published two landmark papers titled “Soluble Substance of Pneumococcal Origin in the Blood and Urine during Lobar Pneumonia” (25) and “The Elaboration of Specific Soluble Substance by Pneumococcus during Growth” (26). Thought initially to have some characteristics in common with proteins, the specific soluble substances of pneumococci of several capsular types and that of a Friedländer bacillus (Klebsiella) were shown by Heidelberger and Avery all to be polysaccharides and the substances comprising the capsules of both species (43). Subsequent studies by Heidelberger and Kendall marked the beginning of quantitative immunology (44).

Recognition by Stryker (85) that loss of capsulation by pneumococci resulted in loss of virulence led Avery to a new potential therapeutic approach, namely, that of enzymatic depolymerization of the pneumococcal capsule in vivo. Avery and Dubos isolated a bacillus from a cranberry bog which produced an enzyme which depolymerized the capsular polysaccharide of pneumococcus type 3 when the latter was provided as its principal source of energy (10). Although the enzyme had no effect on the viability of type 3 pneumococci in vitro or in vivo, it was capable, when injected into infected laboratory animals, of destroying their capsules, rendering them susceptible to phagocytosis and permitting survival of the host (11). Like anticapsular antibody, the enzyme depolymerizing the pneumococcal capsule had no effect on the viability of the organism, however; and in the absence of sufficient enzyme to permit elimination of all viable organisms, capsular regeneration would lead ultimately to the demise of the infected animal. Other potential drawbacks to enzyme therapy were the need for a specific enzyme for each capsular polysaccharide and the effects of antibodies that develop against the enzyme on its activity. Because neither anticapsular antibody nor capsular depolymerizing enzyme has any effect on the viability of infecting pneumococci, their protective effect being to facilitate phagocytosis of the invading organisms, the observation of Rich and McKee that noncapsulated pneumococci caused progressive infection in agranulocytic animals is noteworthy (77). Study of several proteins of the pneumococcal soma, notably pneumolysin, shows them to play a role in its pathogenic properties (17).

Treatment of pneumococcal infections has followed two somewhat parallel courses: immunotherapy and chemotherapy. Indication of the potential utility of immunotherapy had its inception in the observations of the Klemperers, cited above, but its practical application to humans had to await the clear definition of pneumococcal types 1 and 2 by Neufeld and Händel in 1910. A program for the treatment of type 1 pneumococcal pneumonia with
type-specific equine antiserum was initiated at the Rockefeller Institute for Medical Research in 1913 (9). Over the ensuing years, efforts were made to minimize untoward reactions to the antisera by elimination of proteins other than antibodies and to quantify their potency. A unit of antiserum was defined by Felton (28) as “that amount of antibody that will protect at least 50 percent of a series of inoculated standard white mice against 1,000,000 fatal doses of a standard pneumococcal culture of the same type.” The results of treatment of type 1 and type 2 pneumococcal pneumonia with equine antisera show an approximately 50% reduction in mortality among cases of diverse origins collated by Heffron (42). Recognition of differences in the antipneumococcal antibodies of the horse and the rabbit, including the smaller size of the rabbit antibody, led to the introduction in the mid 1930s of rabbit antisera for the treatment of pneumococcal pneumonia caused by a number of capsular types (48). Although type-specific rabbit antisera appeared to have significant advantages over equine antisera in terms of dosage and untoward reactions, their use was to be short-lived, the introduction of sulfonamides and, shortly thereafter, of penicillin leading to their abandonment.

Contemporaneously with the development of serotherapy, the initial investigations of pharmacotherapy were undertaken. In 1911, Morgenroth and Levi reported the protective effect of ethylhydrocupreine (optochin), a derivative of quinine, in mice infected experimentally with pneumococci (66). The following year, in what may have been the first published account of the development in vivo of bacterial resistance to a chemotherapeutic agent, Morgenroth and Kaufmann described the isolation of optochin-resistant pneumococci from animals similarly infected (65), an observation confirmed by the in vitro observations of Tugendreich and Russo in 1913 (88). Optochin was used for a brief period to treat pneumococcal infection in humans; a 20-fold increase in resistance to the drug was observed in patients undergoing therapy (63, 64). Its use was abandoned because of its toxicity to the eye and its limited efficacy. Its only use today is in a laboratory test for the presumptive identification of pneumococci (18).

The introduction of the sulfonamides in the 1930s made available for the first time relatively safe and effective antibacterial drugs. Although sulfanilamide proved ineffective in treating pneumococcal infections, sulfapyridine and a number of successive congeners were moderately successful in the management of pneumococcal pneumonia. Their use marked one of the first times, if not the first time, that levels of drug in the blood were used to regulate dosage (58), and although earlier experience with optochin was not cited, they became other examples of the pneumococcal capacity to develop resistance to antimicrobial agents (56).

The advent of penicillin treatment of both nonbacteremic and bacteremic pneumococcal pneumonia, reducing the overall case fatality rate to 5 to 8%, had a profound effect on professional attitudes toward these infections. Because therapy was effective irrespective of the infecting pneumococcal type, capsular serotyping of the organism was largely abandoned and its recognition declined significantly. Only when typing was reintroduced was it recognized that attack rates of pneumococcal infection had changed little, if at all (8). Although pneumococcal mutants showing incremental resistance to penicillin
had been isolated from experimentally infected mice in 1933 (80), another three decades were to elapse before such mutants were recovered from humans (38), initiating a therapeutic problem of ever-increasing magnitude. Penicillin resistance in the pneumococcus is a result of mutation in one or more of the bacterium’s penicillin-binding proteins (96).

The ability of pneumococci to adjust to exposure to other antimicrobial agents appears subject to few, if any, limitations. Mutants resistant to cephalosporins, tetracyclines, chloramphenicol, macrolides, quinolones, and aminoglycosides have all been recognized (51), and mutants tolerant of vancomycin have also been described (74).

Because of the persisting morbidity and mortality worldwide of pneumococcal infections and the increasing incidence of antimicrobial resistance, a program to reintroduce a polyvalent pneumococcal polysaccharide vaccine was initiated in the 1960s. Attempts to prevent pneumococcal infection date from 1911. Although Sternberg had reported in 1882 that injection of a rabbit with his saliva after its treatment with an antiseptic had rendered the animal immune to what would otherwise have been a subsequent lethal injection of its untreated saliva (84), no one seems to have recognized the prophylactic implications of this experiment.

The stimulus to develop a pneumococcal vaccine arose in the gold mines of South Africa, where pneumonia was epidemic in the early years of the 20th century. Sir Almroth Wright, credited with the development of typhoid vaccine, carried out with his collaborators several trials of pneumococcal vaccines of unknown serotypic constitution involving 50,000 miners (95). Although Wright, who had an antipathy to biostatistics, thought the vaccine to be effective, subsequent analysis failed to support this conclusion. Wright’s protégé, F. Spencer Lister, continued studies in South Africa, described independently the serologic diversity of pneumococcal strains causing infection, and demonstrated that one could inject humans intravenously with 2 to 4 billion heat-killed pneumococci without untoward effect (54). Thirty years later, Heidelberger and his associates were to show that 10 billion pneumococci of types 1 and 2 yielded approximately 30 to 40 μg of capsular polysaccharide (45). Lister carried out several trials of polyvalent vaccines of heat-killed pneumococci and might have shown their efficacy in the second decade of the 20th century had it not been for his choice of controls. Because attack rates of pneumonia were not the same at different mining compounds, the decision to vaccinate all miners at one compound and to compare the attack rate of pneumonia with that in unvaccinated miners at another compound was faulted by the vaccine’s detractors, and the vaccine’s efficacy remained moot (3).

In World War I, several trials of vaccines of whole pneumococcal cells were carried out elsewhere in military and other populations. Reviewed by Heffron, who estimated that a million subjects may have been included in the several trials, the efficacy of vaccines of killed whole pneumococcal cells remained uncertain (41).

Antigenicity of the capsular polysaccharide of pneumococcus type 3 in the mouse was recognized in the late 1920s (79), and in 1931, Francis and Tillett, injecting intradermally small amounts of capsular polysaccharides to determine the adequacy of serum therapy in patients with pneumococcal pneumonia,
discovered that polysaccharide of a type differing from that causing pneumonia stimulated the formation of antibodies, demonstrating thereby the immunogenicity of capsular polysaccharides in humans (32), an observation confirmed by Finland and others (29, 30). These findings led to several inconclusive trials of capsular antigens as vaccines (27), but conclusive evidence of the efficacy of a tetravalent vaccine in a military population experiencing epidemic pneumococcal disease was reported by MacLeod et al. (57). It was shown also in the same trial that if an individual was a carrier of a pneumococcal type represented in the vaccine, vaccination would not eliminate the carrier state, but if the individual was not a carrier, vaccination would reduce by half the likelihood of his becoming one. As a sequel to this study, two hexavalent vaccines were licensed in the 1940s, a time when interest in pneumococcal infection was at its nadir; after several years, they were withdrawn by their manufacturer for lack of use.

Study a decade later revealed that there had been little if any change in the epidemiology of pneumococcal infection despite the introduction of active therapeutic agents and that a significant number of deaths still occurred (8). The findings led to renewed interest in prophylaxis to protect those identifiably at high risk. Accordingly, trials of polyvalent capsular polysaccharide vaccines were carried out in South Africa involving 12,000 young adult males with a high attack rate of disease (7). The aggregate efficacy of a tridecavalent formulation in preventing disease caused by types represented in the vaccine was 78.5% \( (P < 0.0001) \), and its ability to prevent radiologically confirmed pneumonia irrespective of cause in a population in which pneumococcal infection predominated was greater than 50% \( (P < 0.0001) \). On the basis of the foregoing data, a 14-valent vaccine was licensed in 1978, and in 1983, the formulation was expanded to include 23 capsular polysaccharides, the most complex vaccine administered to humans, designed to prevent 23 immunologically distinct infections. A case-controlled study in the United States among immunocompetent persons over 40 years of age with infection of a normally sterile bodily site showed the aggregate efficacy of the vaccine to be 61% \( (P < 0.0001) \), with a more rapid decline in protection among those 65 years of age and older (81).

Although capsular polysaccharides are suitable antigens for adults, they are not immunogenic in the human infant, a fact recognized as early as 1937 (23). The rabbit, like the human infant, is similarly unresponsive to polysaccharide antigens, but as shown by Avery and Goebel (12), if the polysaccharide is linked chemically to a protein, antibodies to the polysaccharide will be made. Seeking to provide an effective vaccine against infection with *Haemophilus influenzae* type b, the most common cause of bacterial meningitis in children under 2 years of age, Robbins et al. coupled the type b capsular polysaccharide to each of several well-characterized proteins and succeeded in producing a safe and effective vaccine (78). These findings led to the production of analogous pneumococcal polysaccharide-protein conjugates which have proved similarly effective in preventing systemic infection with the capsular types predominating as causes of infection in infancy (15).

Several protein antigens of the pneumococcus have been studied for their prophylactic effects in laboratory animals with the aim of developing vaccines
of lesser complexity than those of capsular polysaccharides. To date none has been the subject of field trials in humans, and their prophylactic potential remains to be determined (19).

No discovery arising from the study of bacteria has had a greater impact on biology than the one revealing genes are composed of DNA, the outcome of investigations of the pneumococcus. As noted earlier, noncapsulated pneumococci can often be recovered when capsulated organisms are grown in liquid medium containing homologous anticapsular antibody. In the early 1920s, the British epidemiologist Fred Griffith undertook a study to define conditions under which noncapsulated variants of pneumococcus type 2 would regain capsulation and virulence in vivo (35). His initial protocol entailed injecting mice subcutaneously with living noncapsulated pneumococci derived from a strain of capsular type 2 together with a large amount of vaccine of heat-killed capsulated type 2 pneumococci. From mice dying following inoculation with the foregoing mixture, Griffith recovered virulent capsulated type 2 pneumococci. Extending his study, he repeated the experiment, substituting on this occasion a heat-killed vaccine of type 1 pneumococci, and recovered pneumococci of capsular type 1. Griffith’s controls were meticulous. As noted by Hayes 38 years later in an appreciation of Griffith’s work, “. . . . it is strange that Griffith does not draw attention to, much less stress, what now seems to us the most striking feature of transformation, namely, that it results in an hereditary alteration of character wherein, of course, lies its real biological significance” (40).

Griffith’s findings, published in 1928, were confirmed the same year by Neufeld and Levinthal in Germany (72) and by Dawson at the Rockefeller Institute (24). Further studies led to the successful transformation of pneumococci in vitro (60) and culminated in 1944 in the publication by Avery, MacLeod and McCarty entitled “Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of transformation by a deoxyribonucleic acid fraction from pneumococcus type 3” (13). The report, the first to attribute biological activity to a nucleic acid, was greeted initially with skepticism by some, and acceptance of the role of DNA was not immediate. Purification of DNase initiated by McCarty (59), leading to the ultimate crystallization of an enzyme inactivating the transforming principle, and the transformation of additional pneumococcal attributes brought ultimate acceptance of the genetic properties of DNA (61). The work of Chargaff showing that the adenine-thymine:guanine-cytosine ratios of DNAs from different species differed provided evidence that all DNAs were not alike (20) and played a role in the development by Watson and Crick of their model of the DNA molecule that was to revolutionize genetics (89). It is beyond the scope of this foreword to describe the development of this discipline in the past half century, much of which can be found in the volume entitled *Recombinant DNA* by Watson et al. (90). It is fitting to note, however, that the complete genome of a capsulated pneumococcus was recorded in 2001 (86).

Problems remain. Can currently available polysaccharide vaccines be used more effectively, and will any other antigen(s) of the pneumococcus prove more effective? Will it prove possible to discover antipneumococcal agents to
which the organism is itself unable to give rise to resistant mutants? Perhaps no problem is more important than unraveling the mystery of how pneumococcal infection leads ultimately to the patient’s demise. The problem was stated tellingly by Osler (75) in 1897:

Very large areas of the breathing surface may be cut off without disturbing the cardiovascular mechanism. In no way is this more strikingly shown than by the condition of the patient after the crisis. On one day with a lung consolidated from apex to base, the respirations from 60 to 65 and the temperature between 104° and 105°, the patient may seem in truly desperate condition, and it would appear rational to attribute the urgent dyspnoea and the slight cyanosis to the mechanical interference with the exchange of gases in the lungs. But on the following day, dyspnoea and cyanosis may have disappeared, the temperature is normal and the pulse rate is greatly lessened and yet the physical condition of the lungs remains unchanged. We witness no more striking phenomenon than this in the whole range of clinical work and its lesson is of prime importance in this very question showing that the fever and the toxins rather than the solid exudate are the essential agents in causing cardio-respiratory symptoms. The toxemia outweighs all other elements in the prognosis of pneumonia; to it . . . . is due in great part the terrible mortality of this common disease and unhappily against it we have as yet no reliable measures at our disposal.

Although the advances in antimicrobial therapy of the last century have bought at least temporary respite from the mortality of pneumococcal infection, the interactions of the pneumococcus with its mammalian host still remain, for the most part, to be unraveled. Advances in understanding of the minutiae of sepsis are revealing the enormous complexity of both host and parasite and of their interactions (21). Much remains to be learned from continued study of the pneumococcus and of its interactions with humans. The note of optimism sounded by Benjamin White in his monograph The Biology of Pneumococcus, published in 1938, remains relevant today:

The study of the members of this small group of microorganisms in a subordinate branch of biology is bringing light into some of the obscure realms of the related sciences. The peculiarities of Pneumococcus are yielding a generous return to the investors who have cast in their resources with its lot, resulting in the accumulation of a store of solid bullion for the scientist and for mankind. (94)

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The pneumococcus represents both a vast burden of human disease and an enormous opportunity for medical and basic science discovery. This nemesis has a very long track record as a severe, invasive pathogen despite over a hundred years of medical progress. Current events indicate that the situation is only getting worse. The pneumococcus has pushed biological science to the discovery of DNA, of polysaccharide-based vaccines, of quorum sensing, of peptide-based bacterial communication, and many other basic tenets. It is the quintessential gram-positive pathogen and leads the path of discovery of the pathogenesis of this class of organisms, a group that now outnumbers the gram-negatives. Each time we think we are in control, the pneumococcus teaches us that it can beat any defense. Antibiotic resistance has become rampant and involves multiple drugs to the point that there are pneumococci that severely tax our capability to kill them. As time progresses, this naturally transformable pathogen will continue to harvest hazardous weapons while browsing in the DNA gift shop of the human nasopharynx. Good vaccines have been developed but, unfortunately, have been poorly used. Even when they are used appropriately, the bacterium switches capsular type to evade elimination. We have learned a great deal, but there is a long life ahead for investigators in this field.

The scientific database centered around the pneumococcus has been very rich and has become codified in a series of meetings of investigators in the field, always drawing an international crowd. The group was small in California in 1981 and expanded in Portugal in 1996, a meeting resulting in the publication of a book that is a milestone in the field. Since then, pneumococcal investigators, M.D.’s and Ph.D.’s alike, have met every 2 years just to try to keep up with the massive amount of new data and new problems presented by this pathogen. This book comes only 7 years since the last, but it is virtually entirely new. A major catalyst to this increased information is the publication of sequences of several pneumococcal genomes. We will mine
Preface

These data for years to come. Taking stock of where the field is now is meant to provide a framework of where the biggest needs are for future investigation and, importantly, to show where the basic science can translate into changes in clinical care. Thus, this book emphasizes both the details of the bacterium and all it can do as well as the host response and mechanisms of disease. The pace of discovery is intense and richly rewarding, but seasoned investigators know from experience that the pressure is always on because this pathogen will stand still for no one.

It has been immensely rewarding and challenging to track the developments of the pneumococcus, and there are many to thank for making this book a reality. First, I thank my coeditors, who very actively interacted with the authors to expand and interconnect the components of this story. They demanded excellence and got it. My deepest gratitude goes to the authors. Everyone that I asked to write for this project accepted with enthusiasm, indicating that there was a pent-up desire to report and review a reservoir of new knowledge at this time for this field. Authors contacted colleagues and created liaisons as coauthors to bring in a broad representation of experts in the field. As word of the book spread, some investigators even volunteered as authors. Now that is enthusiasm and collegiality! Helpful comments on the scope of the book were provided by Liise-anne Pirofski, Albert Einstein College of Medicine; Michael Caparon, Washington University School of Medicine; Peter W. Andrew, University of Leicester; Larry S. McDaniel, University of Mississippi; and Russell W. Steele, Children’s Hospital, New Orleans. The guidance behind the project came with the experienced and steady hand of Greg Payne at the American Society for Microbiology (ASM). He kept us true to time and worked through expansion of the book as more and more information came from more and more authors. ASM worked tirelessly even over the December holidays to assemble the final book. Finally, I would like to acknowledge that my energy to continue to read, write, and edit was renewed every day by my very industrious lab people, by the faculty in Infectious Diseases at St. Jude Children’s Research Hospital, and by my crack-erjack children, Laura and Stefan.

My hope is that this book will serve as a milestone in the race against this pathogen and invigorate young investigators in the field. Those of us in the “senior investigator group” all remember key people, now giants in biomedical science, who encouraged us early on in our careers. I hope that when new investigators of gram-positive microbiology and pathogenesis come to us for advice, we will reach up to the bookshelf and take down this book, enthusiastically saying, “This is a great place to start.”

Elaine Tuomanen
January 2004
24. NEW VACCINES: BASIC SCIENCE DEVELOPMENTS

Assessment of their protective efficacy has been carried out in different laboratories, using a variety of animal models and challenge strains. Clearly, a comprehensive series of direct comparative protection studies needs to be performed in order to determine which of these proteins provides the strongest protection against the widest variety of \textit{S. pneumoniae} strains.

**Combination Protein Vaccines**

Virtually all of the pneumococcal proteins under consideration as vaccine antigens are directly or indirectly involved in the pathogenesis of pneumococcal disease. Mutagenesis of some combinations of virulence factor genes, for example, those encoding pneumolysin and either PspA or PspC, or both, has been shown to synergistically attenuate pneumococcal virulence in animal models, implying that the respective proteins function independently in the pathogenic process (7, 12). This strongly suggests that immunization with combinations of these antigens might provide additive protection. Moreover, there may be differences in the relative protective capacities of the individual antigens against particular \textit{S. pneumoniae} strains, particularly for surface-exposed antigens that exhibit some degree of sequence variation. Thus, a combined pneumococcal protein vaccine may elicit a higher degree of protection against a wider variety of strains than any single antigen. To date, only a limited number of combination experiments have been performed. Immunization of mice with a combination of the pneumolysoid PdB and PspA provided significantly more protection against intraperitoneal challenge than immunization with either protein alone. However, combining either protein with PsA did not result in enhanced protection (81). The potential benefits of combination protein vaccines are also well illustrated using a recently developed mouse model of nonbacteremic pneumonia, which closely reflects the most common form of pneumococcal respiratory disease in humans (18). In this system, subcutaneous immunization (using alum adjuvant) with either PdB or PspA, but not PsA, significantly reduced numbers of \textit{S. pneumoniae} bacteria in the lungs 7 days after challenge (Fig. 1). A significant additional reduction in bacterial load was achieved by immunization with a combination of PdB and PspA, but not when either protein was combined with PsA (Fig. 1) (18). These findings contrast with those obtained using mucosal (intranasal) immunization with the same proteins with CTB as adjuvant. As discussed above, immunization with either PspA or PsA, but not PdB, reduced the level of carriage of \textit{S. pneumoniae} after intranasal challenge, and the combination of PspA and PsA was more effective than either antigen alone (16). These findings imply that PsA is either more important for survival of \textit{S. pneumoniae} in the nasopharynx than in the lung or that it is more accessible to exogenous antibody in the former niche. On the other hand, pneumolysin appears to play only a minor role during the colonization phase but is clearly important once the organism has been aspirated into the lungs. Thus, optimum vaccine formulation will be dependent upon the mode of vaccine delivery and the stage of the pathogenic process being targeted for immunoprophylaxis.

To date, only a limited number of other pneumococcal protein combinations have been tested for additive protective immunogenicity. Immunization with both the iron transporters PiuA and PiaA was more effective than either antigen alone (23). PdB and PspC have also been tested in combination, but in that study, PspC imparted such strong protection against intraperitoneal challenge on its own that additional protection was not evident when combined with PdB (83). Clearly, additional comparative studies of the protective efficacy of the better-characterized proteins, as well as the more recently identified vaccine candidates (both singly and in combination), are required to enable informed decisions on the formulation of a protein-based pneumococcal vaccine.
Consideration should also be given to using protein antigens as supplements to PS-protein conjugate vaccines. Incorporation of one or more proteins common to all \textit{S. pneumoniae} serotypes may significantly reduce the problems associated with limited serotype cover and replacement carriage associated with the conjugate vaccines, although the problem of high cost remains. Pneumolysoid has also been proposed as an alternative carrier in PS-protein conjugate vaccines, and conjugates of PdB with type 19F PS have been shown to be highly immunogenic and protective in mice (61, 90). In a more recent study, a similar detoxified pneumolysin derivative was shown to be a very effective carrier protein in a tetravalent conjugate vaccine formulation including PS types 6B, 14, 19F, and 23F (72). Use of pneumolysoid, or other suitable pneumococcal proteins, as carriers for PS in conjugate vaccines may also minimize any problems associated with overuse of existing carrier proteins.

MUCOSAL VACCINATION STRATEGIES

Given the pivotal role of nasopharyngeal colonization in the transmission of \textit{S. pneumoniae} and as a precursor of pneumococcal disease, vaccination strategies specifically designed to elicit mucosal immune responses may be more efficacious than parenteral immunization for certain antigens, particularly those implicated in colonization. To date this has been examined in animal models using direct intranasal administration of vaccine formulations (killed whole cells or purified antigens) with a strong mucosal adjuvant such as cholera toxin (CT), the related \textit{E. coli} heat-labile enterotoxin (LT), or cytokines such as interleukin-1, interleukin-12, or granulocyte macrophage colony-stimulating factor (3, 113). Use of CT and LT holotoxins as adjuvants in human vaccine formulations is somewhat controversial, owing to their reactogenicity. However, significant mucosal adjuvant activity resides in the B sub-

![Figure 1](image.png)

**FIGURE 1** Protection against pulmonary infection with \textit{S. pneumoniae} elicited by immunization with PspA, PdB (genetically toxoided pneumolysin), PsaA, or combinations thereof. CBA/N mice were immunized with the indicated proteins on alum or with alum alone, challenged with $10^{6.89}$ CFU of \textit{S. pneumoniae} strain EF3030 (capsular group 19), and sacrificed 7 days later to determine numbers of CFU in their lungs. Significance of difference relative to control mice (alum only): *, $P < 0.04$; **, $P < 0.001$. (Reproduced with permission from the \textit{Journal of Infectious Diseases} [18].)
units of CT and LT, and these are much less reactogenic, although there are residual concerns because of their capacity to bind to GM1 receptors on olfactory nerve endings.

Intranasal administration of heat-killed type 4 pneumococci resulted in strong humoral and mucosal responses to type 4 PS and protection from homologous challenge (47). Similar anti-PS responses in mice have been achieved using purified PS conjugated to either CTB or an LT derivative (49, 102). On the other hand, use of killed nonencapsulated pneumococci has been shown to prevent nasopharyngeal carriage of type 6B pneumococci in a mouse model and to protect rats from intrathoracic challenge with virulent type 3 pneumococci (66). This study is a further demonstration of non-serotype-dependent protection achieved using non-PS antigens. The protective efficacy against carriage of mucosal immunization with purified PspA and/or PsaA in the presence of CTB has already been discussed (16). In a more recent study, coencapsulation of PsaA and CTB within alginate microspheres was shown to elicit higher serum and mucosal immune responses after oral immunization of mice than did nonencapsulated antigens, and this translated into stronger protection against intranasal challenge (101).

An alternative means of eliciting mucosal immune responses involves oral administration of live recombinant carrier bacteria expressing pneumococcal antigens. Recombinant attenuated salmonellae expressing PdB (92), PspA (54, 79) or both of these antigens as well as PsaA (E. M. Barry, A. R. Santiago, J. Sampson, E. Ades, G. Carlone, D. Briles, J. Paton, and M. Levine, Abstr. 3rd Int. Symp. Pneumococci Pneumococcal Dis., p. 102, 2002) have been constructed and shown to elicit mucosal and humoral antibody responses in mice after oral immunization. Furthermore, for strains expressing PspA, protection against systemic challenge was also demonstrated (54, 79). Expression of type 3 PS has also been achieved in Lactococcus lactis, which has been proposed as an alternative carrier for vaccine antigens (38). However, the mechanism of biosynthesis of this PS serotype is much simpler than that of all the other clinically significant pneumococcal PS types and requires expression of only a small number of genes (91). Expression of the other much larger PS biosynthesis loci in heterologous bacteria may be extremely difficult, and any such live vaccines would also suffer from the disadvantages of serotype-dependent protection and poor immunogenicity of PS antigens in high-risk groups.

DNA VACCINES
A further strategy under consideration for prevention of pneumococcal disease is the use of DNA vaccines. This involves introduction of naked plasmid DNA carrying genes encoding protective antigens under the control of a eukaryotic promoter, either by intramuscular injection or transdermally using a gene gun. The naked DNA is taken up by host cells and the antigens are expressed in vivo. This approach has been used for a variety of pathogens and is attractive because DNA vaccines are potentially cheap to produce on a large scale. They usually elicit both humoral and cell-mediated immune responses, and although protection against S. pneumoniae is generally considered to be antibody dependent, the role (if any) of cell-mediated immune responses has not been investigated to any significant extent. One study reported construction of a DNA vaccine plasmid encoding the alpha-helical N-terminal half of PspA (the region which contains the cross-protective epitopes). This induced strong antibody responses in mice and conferred long-lasting protection against both homologous and heterologous S. pneumoniae challenge strains (15). However, in another study, immunization with constructs directing expression of similar regions of PspA from different S. pneumoniae strains resulted in production of cross-reacting antibodies, but protection against challenge was confined to strains expressing related PspA types (75). The same group also examined DNA vaccine constructs directing expression of either the C-terminal two-thirds of PspA or full-length PsaA,
which elicited significant antibody responses to the respective protein in mice (73). However, protection against challenge was not elicited by the C-terminal PspA-expressing construct in a subsequent study (74), while that for the PsA-expressing construct has not been examined.

Use of DNA vaccine delivery systems for PS antigens is extremely problematic, not only because of the multiplicity of serotypes but also because the genetic loci encoding PS biosynthesis are very large, comprising up to 20 or more genes for each PS type (91). An innovative solution to the latter problem has been achieved using an anti-idiotype approach. Firstly, a monoclonal antibody to type 4 PS was used to screen a phage display library, and this identified a peptide mimic capable of eliciting an anti-type 4 PS response. An oligonucleotide encoding this peptide was then inserted into a DNA vaccine vector and this elicited an anti-type 4 antibody response in mice (62). It remains to be seen whether such antibodies are protective against challenge with type 4 pneumococci, and whether peptide mimics can be developed for a sufficient number of the other PS serotypes.

CONCLUSIONS
The ongoing high global morbidity and mortality associated with pneumococcal disease, and the complications caused by increasing rates of resistance to antimicrobials, have underpinned extensive efforts in recent years to develop more effective vaccination strategies against \textit{S. pneumoniae}. These efforts have benefited from a better understanding of the mechanisms of pathogenesis of pneumococcal disease and the advances made possible by the advent of recombinant DNA technology and access to genome sequence data. The polyvalent PS vaccines have doubtless prevented a large number of deaths from invasive disease in recipients belonging to those patient groups for whom this vaccine is currently recommended. The newer PS-protein conjugate formulations will also confer a very high degree of protection on young children against included serotypes, and they may also have an impact on prevalence of drug-resistant strains. However, there is now general acceptance that this vaccination approach is not without its drawbacks, and as explained above, the initially substantial clinical benefits that are expected to be derived from widespread use of conjugate vaccines may diminish with time. It will take many years for the overall impact of conjugate vaccines on disease burden and the population biology of \textit{S. pneumoniae} to become apparent. At the very least, use of the conjugate vaccines will buy time for development of cheaper, non-serotype-specific vaccines based on combinations of protein antigens. It must be emphasized, however, that the success of these protein vaccines is not dependent upon real or perceived failure of the conjugates. Rather, the two approaches should be viewed as complementary, each having an important role to play in global prevention of pneumococcal disease. Neither should development of parenteral protein vaccines impede future research on mucosal or DNA-based delivery systems, which may further improve presentation of protective antigens to the immune system, thereby optimizing host responses.

REFERENCES


SUBJECT INDEX

Acquisition, duration of carriage, and invasive disease, 142, 143
risk factors for, 139–140
Adhesins, 171
Adults, antibiotics for infection in, 326
bacteremia in, antibiotics for, 319–320
meningitis in, 324–325
pneumonia in, 321–322
polysaccharide vaccines and, 406–407
Age, as risk factor, 154
carriage and, 138–139
Alcohol use, as risk factor, 157
Allelic replacement, 5
Alveoli, epithelium of, damage to, 227
bacterial toxins and, 227–228
invasion into, CP230
Amino acid chirality, 194
Aminoglycosides, 284
Amoxicillin, 332, 334
oral, in otitis media, 316
resistant strains and, 288, 289–291
Amoxicillin-clavulanate, in otitis media, 315–316
nasopharyngeal carriage and, 295–296
Ampicillin, 332
Antibiotic resistance, clones and, 131
determinants of, genetics and, 335–357
in infection, clinical relevance of, 331–338
meningitis and, 334
reduction of, effect of vaccines on, 305–307
Antibiotic-resistant strains, carriage of, 140, 141
Antibiotics, and vaccines, changing ecology with, 283–313
consumption of, and antibiotic resistance, 296–297, CP297
direct effects of, 284–297
effects on carriage during treatment and follow-up, 288–296
for bacteremia in adults, 319–320
for infection
in adults, 326
in children, 325
in otitis media, “double-tap” tympanocentesis and, 314–315
interactions of, mechanisms of, 304–305
new, and effects of conjugate vaccines on resistance, 359–360
selective effects of, interactions between, 304–307
use of, population-biological considerations for, 286–287
Antibodies, adaptive immunity mediated by, 367–368
defects of, 256–259
development of, in response to carriage, 142–143
mechanisms of protection by, 367
natural or vaccine-induced, effect on carriage, 143–144
Antigen A, pneumococcal surface, and metal-binding lipoproteins, 391–392
Antigen-driven T-cell-independent response, costimulation of, 369–370
Antigen-preventing cells, 368
Antigens, polysaccharide, 403
Antimicrobial agents, resistance to, varying by area, 296–297
Antimicrobial peptides, 174
Antipolysaccharide, differential regulation of, 368
Antiprotein Ig responses, differential regulation of, 368
Apoptosis, in dendritic cells, 375–376
Arthritis, rheumatoid, 217
septic, 217
SUBJECT INDEX

Autolysin(s), 7–8, 49
"true" pneumococcal, 75–76

Azalides, 331
Azithromycin, 285, 334
resistance to, 293, 294, 295

Background, 3–4
Bacteremia, in adults, 319–320
antibiotics for, 319–320
in children, 317–318
pneumolysin and, 65, 67
primary, 211, 216
secondary, 211
since 7-valent conjugate vaccine licensure, 318–319

Bacteria, in nasopharynx, prediction of eradication of, 284–286
pathogenic and nonpathogenic, walls of,
proinflammatory activities of, 194–195
species of, in upper respiratory tract, 283

Bacterial murein hydrolases, 75
pneumococcal, 75

Bacterial toxins, damage to epithelial alveoli and,
host response to, 227–228

Bacteriophages, encoding, cell wall lytic enzymes and, 76–84

Bacterium-bacterium interactions, 176–177
B-cells, marginal-zone, and B-1 cells, and T-cell-independent response, 368–369

Beta-lactams, 284, 331–333
resistant strains and, 288, 289–291, 333, 350
penicillin-binding proteins and, 339–340, 341
 tolerance associated with, 340–342
Block-type (Wzy-dependent) pathway, 35–38

Blood-brain barrier, anatomy of, 240, 242
transmigration across, 240, 242

Brain, barriers to, 240–241
Brochitis, chronic, exacerbations of, 333
Bronchopneumonia, 221, 223
activities of pneumolysin and, 67

BURST, 121–123

Cancer, and transplantation, 262–263
Capsular genes, transformation and, 109–110
Capsular polysaccharides, 30

Capsule(s), 30–48
historical perspective on, 30–32
inhibitory effects of, 172
structures of, repeating units of, 30, 31
synthesis of, mechanisms of, 35–39
 regulation and modulation of, 39–40
types of, exchanges of, 32

Capsule loci, genetic exchange of, 35
genetic organization of, 32–35
Carriage, 136–147, 211, 407–408
as least invasive infection, 412–413
definition and measurement of, 136–138
duration of, acquisition, and invasive disease, 142, 143
effect of natural or vaccine-induced antibodies on, 143–144
effect of vaccines on, 297–304
evidence for, 299–302
effects of antibiotics on, during treatment and follow-up, 288–296
efficacy of conjugate vaccines against, 411
historical background of, 138
host specificity and, 169–170
mechanisms of, 169–182
of antibiotic-resistant strains, 140, 141
response to, development of antibodies and, 142–143
risk factors for, 138–139
versus disease, 127–129
Carried serotypes, distribution of, 140–142
CbpA, adhesin, 225
domain structure of, 225, 227
interference with C3 binding by, 205–206

Cefazolin, 332
Cefdinir, in otitis media, 316
Cefotaxime, 332, 334
resistance to, 342, 346

Ceftriaxone, 332, 334
intramuscular, in otitis media, 316

Cefuroxime, 332
Cefuroxime axetil, in otitis media, 316

Cephalosporins, 285, 342
in bacteremia, 317–318
resistant strains and, 288, 289–291

Chemokines, 187, 243

Children, antibiotics for infection in, 325
bacteremia in, 317–318
invasive disease in, 256, 258
meningitis in, steroids in, 323–324
therapy for, 322–323
otitis media in, 314–316
polysaccharide vaccines and, 407
prevention of, 317
pneumonia in, 320
polysaccharide vaccines and, 407

Chloramphenicol, 354
Choline, requirement for, 173
Choline-binding domain (C-LytA), 82
Choline-binding proteins, 49–60, 390–391
attachment of, 51–53
biological function of, 54–55
and relevance to disease, 54–55
C3 binding and, 205
cell wall hydrolases as, 80–81
expression of, regulation of, 54
functions of, 49
immunology of, 56–57
pneumococcal, 54
structural features of, 49–51
Chromosomal transformation, 92–93
Circular integration, in circular synopsis, 94
Clindamycin, 284
Clinical syndromes, pathogenetic categorization of, 211–220
Clones, and clonal complexes, identification of, 120–123, CP123
antibiotic resistance and, 131
diversification of, mechanism and rate of, 123
international invasive, 131
invasive molecular, epidemiology of, 153
major, causing invasive disease, 129–131
serotypes and, 125–127
Cloning, and chromosomal facilitation of plasmid transfer, 94
Colonization, pathways for, 176
versus infection, requirements for, 170
Combox regulon, competence for transformation and, 97–99
Comparative genome hybridization (CGH), 23–26, CP23
Competition, for transformation. See Transformation, competence for
Complement, defects of, 259–260
evasion of, 174
virulence, phagocytosis, and protection of cell surface, 41–42
Complement-mediated functions, proteins interfering with, 204–208
Complement-mediated opsonization and phagocytosis, biochemistry of, 203–204
Complement pathway(s), 201
classical, proteins, pneumolysin and, 206–207
deficiencies of, and epidemiology of infection, 201–203
proteins of, interactions with, 201–210
Complement receptors, 260
Conjugate vaccines, 385–387
efficacy of, 414
against carriage, 411
against otitis media, 410–411
protective immunity induced by, 408–411
Conjugative transposons, 94
Conjunctivitis, 216
Control, 3
C-reactive protein, 174
Culture technology, measurement and, 137
Cytokines, and immune response, 243
cell-secreted, 187
in humoral immune response, 376–377
production of, 229, 230
Cytoplasmic nod receptors, 184–185, 186
Dendritic cells, 373–374
activation of, 374
antiplysaccharide and antiprotein Ig isotype responses and, 374–375
apoptosis in, 375–376
Detection, 4
Dexamethasone, in meningitis, 323–324, 325
Diabetes mellitus, 263
Diagnostics, molecular, 5–7
Disease, epidemiology of, 148–168
invasive, duration of carriage, and acquisition, 142, 143
major clones causing, 129–131
trends in, 158
DNA, as transforming principle, 90
bound to protein, 102
donor release of, 102–103
facilitation of recombination and, 102
fate within cell, and recombination, 102–105
mismatch repair and, 103–105
molecular fate of, in transformation, 91–92
structure of, 90–91
uptake of, molecular mechanism of, 99–102
DNA vaccines, 395–396
Drugs, multidrug resistance and, 292–296
Eclipse, and entry nuclease, 91–92
Electrophoresis
multilocus enzyme, 120
pulsed-field gel, 119–120
Empyema, as complication of pneumonia, 216
beta-lactam resistance and, 333
Endocarditis, 211–212
penicillin resistance and, 334
Endometritis, 216
Entry nuclease, eclipse and, 91–92
Enzymes, extracellular, 17
Epidemiological methods, 149–150
Epidemiology, of disease, 148–168
trends in, 158
Erythromycin, 350
Evolutionary and population biology, 119–135
Extracellular enzymes, 17
Factor C2 deficiency, 259
Factor H, absorption of, PspC in, 206
Fluoroquinolones, 284, 331, 333, 350, 353–354
Gene expression, regulation of, 170
Gene order, non-conserved, major regions of, 20, 21
Gene redundancy, and functional analysis, 103
Gene transfer, horizontal, 4–5
mosaic genes and, 342–344
Genes, capsular, transformation and, 109–110
encoding proteins, targeting of, 392–393
mosaic, gene transfer and, 342–344
virulence, 108–109
Genetics, 264–265
determinants of antibiotic resistance and, 335–357
population, 5, 6
transformation in, 109
Genome plasticity, 15–27
Genomes, complete, comparisons, 18–22, CP18
features of sequenced strains, 16–17
hybridization, comparative (CGH), 23–26, CP23
organization of, 16–17
Genomics, comparative, 15–27
Genotypes, diversification of, rate of, 123
Glycoconjugate receptors, 171–172
Gram-positive organisms, inflammation induced by,
188–189
inflammatory subcomponents of walls of, 190–193
Growth requirements, 172
Herd protection, by vaccines, 302–304, 306
History, 89–91
Host defense, inflammation and, 183–200
innate, interactions with, 173–175
Host factors, in disease, 154–158
Host structures, adherence to, 170–172
Human immunodeficiency virus (HIV), 217, 254, 405
HIV-1-associated immunodeficiency, 266
HIV-1 infection, 265–267
Humoral immune response, 367–381
Hyaluronic acid, 68
Hyaluronidase, 68
Hydrogen peroxide, 69
Hydrolases, cell wall. See Cell wall hydrolases
Hypogammaglobulinemia, 262
Identification, 4
alternative approaches to, 8–10
Immune response, humoral, 367–381
Immune responses, mucosal, 394–395
Immunity, adaptive, evasion of, 175–176
mediated by antibody, 367–368
innate, components of, 184–188
protective, induced by conjugate vaccines, 408–411
polysaccharide vaccines in, 404–408
vaccine-induced, 403
 Immunocompromised persons, vaccine for, 261, 262
Immunocompromising diseases, 263
Immunodeficiency, and invasive disease, 252–280
impact of, 252–256
Immunogens, subcutaneous, 393, 394
Immunoglobulin G, antipolysaccharide responses, 369
antiprotein responses, differential regulation of, 368
anti-specific response, T cells in, 370–371
effects of vaccines and, 298
Immunoglobulin isotypes, 367–368
Infection(s), antibiotic resistance in, clinical relevance
of, 331–338
carriage as least invasive, 412–413
colonization versus, requirements for, 170
epidemiology of, deficiencies of complement
pathways and, 201–203
hematogenous, 211–212
in children, antibiotics in, 325
invasive, polysaccharide vaccines and, 404–406
joints, bones, and soft tissues, 217
ocular, pneumolysin and, 65
pathogenesis of, pneumolysin in, 65–67
preceeding, as risk factors, 158
respiratory. See Respiratory infections
therapeutic strategies and pitfalls, 314–330
viral, effects of, 177
Inflammation, and host defense, 183–200
Inflammatory bacterial structures, compared, 189–190
Inflammatory signaling, 175
Interspecies recombination, 123–125
Intrastrain diversity, 15–27
Iron, acquisition of, 173
Ketolides, 333, 359
Lactic acid group members, comparisons, 18, 19
Leukocytes, 243–244
migration of, chemokines and, 228–230
response to bacterial toxins, 228
Lipopolysaccharide-binding protein, 187–188
Lipoproteins, 68–69
metal-binding, PsaA and, 391–392
Living conditions, as risk factor, 156–157
LPXTG-anchored surface proteins, 67–68
Lung, virulence determinants in, 225–227
Lymphomas, 263
LytA amidase, structure-function relationships of,
81–82, CP82
LytA autolysin, in pneumococcal infection, 84
lytA gene, 76–78
lytB gene, 78–79
lytC gene, 79–80
Lytic enzymes, cell wall, and encoding
bacteriophages, 76–84
Macrolides, 284, 333, 350
resistance to, molecular basis for, 351–353
resistant strains and, 288, 289–291
Mannose-binding lectin, 201–202
Marginal-zone B-cells and B-1 cells and T-cell-
dependent response, 368–369
Medical conditions, as risk factor, 157
Meningitis, 217, 322
antibiotic resistance and, 334
bacterial animal models of, 239–240
clinical features of and prognosis in, 239
epidemiology of, 238–239
Gram stain of cerebrospinal fluid in, CP239
pathogenesis of, 238–251
in adults, 324
steroids in, 324–325
in children,
steroids in, 323–324
therapy for, 322–323
neuronal injury in, 245, 246
pneumococcal, complication of, 65
pneumolysin and, 66
Mesodiaminopimelic acid, 195
Metabolism, 17
Methionyl-tRNA synthetase transformation, 110
Microflora, competition with, 176–177
Mismatch repair, DNA and, 103–105
Molecular basis, of resistance, 351–355
Molecular diagnostics, 5–7
Mosaic genes, gene transfer and, 342–344
Multidrug resistant strains, 292–296
Multilocus sequence typing, 120
alleles at loci of, 124
Multiple myeloma, 262
Mutagenesis, insertion-duplication, 84
Mutations, in penicillin-binding proteins, 344–346
NADH oxidase, 69
Nasopharyngeal carriage. See Carriage
Nasopharynx, as ecologic niche, 136
eradication of bacteria in, prediction of, 284–286
Neuraminidase, 68
Nonconserved gene order, major regions of, 20, 21
Nontypeable pneumococci, 4
Ocular infections, pneumolysin and, 65
Osteomyelitis, 217
Otitis media, 151, 212, 301, 302, 303, 306–307, 334, 386
antibiotics in, “double-tap” tympanocentesis and, 314–315
efficacy of conjugate vaccines against, 410–411
in children, 314–316
polysaccharide vaccines and, 407
prevention of, 317
inflammation process in, 412
Oxidative stress, 172–173, 244
Pathogen-associated molecular patterns, 183, 185
Pathogenicity islands, stable, 8
Pattern recognition molecule, 242
Pen gene, 80
failure in bacteremia prophylaxis, 334–335
resistance to, 292–293
mechanisms for, 339–349
Penicillin-binding proteins. See Protein(s),
penicillin-binding
Peptides, antimicrobial, 174
stem, monomers and multimers of, 193
structures of, 193–194
Peptidoglycan
structures of fragments of, 192–193
TNF-releasing activity of, 193–194
Peptidoglycan-recognizing proteins, 185–186
Pericarditis, 217
Peritonitis, 217
Phagocytosis, virulence, and complement, and protection of cell surface, 41–42
Phase variation, 39
Phylogenetic relationships, 4–5
Plasmid transfer, chromosomal facilitation of, cloning and, 94
transformation and, 94
Plasticity, genome, 15–27
Platelet-activating factor, and receptor, 188
Pneumolysin, 7–8
activation of complement system, 62
and virulence proteins, 61–74
as pore-forming toxin, 61
as vaccine antigen, 388–389
effects on gene expression, 63–64
effects on mouse macrophages, 63
genetically toxoided, immunization with, 393, 394
in apoptosis, 64
in bacteremia, 65, 67
in infection, 66, 67
in membrane insertion, 62
in pathogenesis of infection, 65–67
inflammatory properties of, 62–64
interaction with animal tissues, 64–65
interaction with neutrophils, 63
lytic activity of, 66
meningitis and, 64, 66
ocular infections and, 65
phospholipase A, activation by, 63
pore-forming toxin, structure-function relationships in, 61–62, CP62
structure-function relationships of, 61–62
undecapeptide region of, 62
Pneumonia, 211, 213–216
antibiotic resistance and, 331–333, 350
complications of, 216
diagnosis of, 222–223
diagnostic microbiology in, 215–216
efficacy of conjugate vaccines against, 409–410
experimental models of, 223, CP223
in adults, 321–322
polysaccharide vaccines and, 406–407
in children, 320
polysaccharide vaccines and, 407
inflammation process in, 412
kinetic events in, 228, 229
laboratory findings in, 215
Pneumonia (continued)
progression of respiratory infections to, 224–225, 226, CP224
radiographic findings in, 214–215
symptoms and physical findings in, 214, 221–222

Polysaccharide antigens, 403
Polysaccharide (PS)-based-protein conjugate vaccines, 382, 385–387
Polysaccharide (PS)-based vaccines, purified, 383–385
Polysaccharide vaccines, efficacy of, 267, 268, 414
in pneumonia in adults, 406–407
in pneumonia in children, 407
in protective immunity, 404–408
invasive infection and, 404–406
mechanisms of protection by, 411–414
Polysaccharides, 368
capsular, 30
Population genetics, 5, 6
transformation in, 109
Protection, correlates or surrogates of, 413–414
Protein A, pneumococcal surface, vaccine potential of, 389–390
Protein(s), 368
as vaccine antigens, 393–394
C-reactive, 174
choline-binding. See Choline-binding proteins
DNA bound to, 102
genes encoding, targeting of, 392–393
interfering with complement-mediated functions, 204–208
lipopolysaccharide-binding, 187–188
LPXTG-anchored surface, 67–68
of classical complement pathways, pneumolysin and, 206–207
of complement pathways, interactions with, 201–210
penicillin-binding, 339
beta-lactam resistance and, 339–340, 341
beta-lactam resistance and, 339–340, 341
-independent mechanisms of beta-lactam resistance, 346
mutations in and resistance, 344–346
recombinant pneumococcal, in degradation of C3, 207
surface-exposed, 17–18
PspA, inhibition of complement activation by, 204–205
PspC, in absorption of Factor H, 206
Purified protein vaccines, 387–394
Quinolones, resistance to, mechanisms of, 353–354
Quinupristin-dalfopristin, 359–360
Quorum sensing, competence systems for, transformation and, 110
in competence for transformation, 95–97
Recombinant pneumococcal protein, in degradation of C3, 207
Recombination, DNA fate within cell and, 102–105
facilitation of, DNA and, 102
interpecies, 123–125
Repeats, genome evolution and, 26
gene-wide density of, 26
Replacement, 299–301
Resistance, determinants of, diversity of, 357–359
effects of conjugate vaccines on, new antibiotics and, 359–360
molecular basis of, 351–355
penicillin-binding proteins and, 344–346
Respiratory infections, clinical features of, 221–222
diagnosis of, 222–223
epidemiology of, 221
experimental models of, 223, CP223
localization to alveoli in, 224
pathogenesis of, 223–224
progression to pneumonia, 224–225, 226, CP224
risk factors for, 221
Respiratory tract, attachment and invasion of, 221–237
upper, bacterial species in, 283
Restriction, transformation and, 103–105
Restriction enzyme systems, 103, 104
Restriction systems, transformation and, 109
Rifampin, 355
Salpingitis, 216
Sampling, interval, measurement and, 137
“Serotype replacement,” 299
Serotypes, clones and, 125–127
epidemiology of, 150–153
Sex, race and ethnicity, as risk factors, 155–156
Sickle cell disease, 261–262
Sigma factor, alternative, competence for transformation and, 97
Signal transduction systems, two-component, 106–107
and virulence, 107
Signaling, inflammatory, 175
Signaling events, 242–243
Sinusitis, 212–213
cotrimoxazole prophylactic therapy and, 333
Socioeconomic status, as risk factor, 156
Spleen, dysfunction of, splenectomy and, 260–261
Splenectomy, and splenic dysfunction, 260–261
Steroids, in meningitis
in adults, 324–325
in children, 323–324
Strain variation, constitutive competence for transformation and, 99
Streptogramins, 333
Stress, 264
Sulfamethoxazole, 355
Superoxide dismutase, 69
Surface-exposed proteins, 17–18
Synercid, 359–360
Synthase-dependent pathway, 37, 38–39
T cells, in anti-specific Ig response, 370–371
major inductive role for, 371
T-cell costimulation, requirement for, 371–372
T-cell help, cognate versus noncognate, delivery of, 372–373
T-cell-independent response, antigen-driven, costimulation of, 369–370
marginal-zone B-cells and B-1 cells and, 368–369
Telithromycin, 359
Tetracycline, 333, 354
Ticarcillin, 332
Tobacco use, as risk factor, 157–158
Tracheobronchitis, acute purulent, 213
Transduction systems, signal, two-component, 106–107
and virulence, 107
Transformation, 89–115
and competence systems for quorum sensing, 110
and mutational analysis of function, 106
capsular genes and, 109–110
cassette mechanisms and, 109–110
chromosomal, 92–93
comparison with other gram-positive and gram-negative species, 105–106
competence for, alternative sigma factor, 97
and regulation of, 94–95
combox regulon and, 97–99
constitutive, strain variation and, 99
quorum sensing in, 95–97
quorums versus crowds and, 109
discovery of, 90
DNA and, 90
genes in, 100
genetic, 89
modes of, 92–94
in population genetics, 109
methionyl-tRNA synthetases, 110
molecular fate of DNA in, 91–92
natural, 89
plasmid transfer and, 94
quantitation of, 90
related to other regulons and virulence, 106–109
release of donor DNA and, 101–102
restriction and, 103–105
restriction systems and, 109
Transmission, 170
Transplantation, cancer and, 262–263
Transport, 17
Transposon(s), composite, 358
conjugative, 358
conjugative, 94, 357–358
elements related to, 358–359
Trimethoprim, 355
Trimethoprim-sulphamethoxazole, 284, 331, 333, 334, 350, 354
resistant strains and, 288, 289–291
Tymanocentesis, “double-tap,” and antibiotics in otitis media, 314–315
Vaccination strategies, mucosal, 394–395
Vaccine(s)
antibiotics and, changing ecology with, 283–313
interactions of, mechanisms of, 304–305
selective effects of, interactions between, 304–307
antigens, proteins as, 393–394
available at end of 2003, 404
conjugate, 385–387
effects on resistance, new antibiotics and, 359–360
efficacy of, 414
against carriage, 411
against otitis media, 410–411
protective immunity induced by, 408–411
DNA, 395–396
effect on antibiotic resistance, 305–307
effect on carriage, 297–304
evidence for, 299–302
efficacy of, 267–269
for immunocompromised persons, 261, 262
future prospects for, 304
herd protection by, 302–304, 306
immunity induced by, 403
new, need for, 382–383
poly saccharide, efficacy of, 414
in pneumonia in adults, 406–407
in pneumonia in children, 407
in protective immunity, 404–408
invasive infection and, 404–406
mechanisms of protection by, 411–414
poly saccharide-based (PS)-protein conjugate, 382, 385–387
polyvalent poly saccharide-based (PS)-protein conjugate, 387
purified poly saccharide-based (PS), 383–385
purified protein, 387–394
7-valent conjugate, protective efficacy of, 408–409
7-Valent conjugate vaccine licensure, bacteremia since, 318–319
Viral infection, effects of, 177
Virulence, capsule in, colonization and adherence of, 40–41
capsule type in, role of, 41
complement, phagocytosis, and protection of cell surface, 41–42
determinants of, in lung, 225–227
nature of, 40–42
two-component signal transduction systems and, 107
Virulence genes, 108–109
Xylitol, in prevention of otitis media, 317
Zinc metalloproteases, 69