or complement activation properties (44). Immunization of mice with a pneumolysoid (PdB) carrying a Trp_{433}-Phe mutation (resulting in >99.5% reduction in cytotoxicity) provided a significant degree of protection against all nine capsular serotypes of *S. pneumoniae* tested (45). Humans develop antibody to pneumolysin as a result of natural exposure to *S. pneumoniae*, and the elicited antibody can passively protect mice from pneumococcal disease (46). Thus, it is anticipated that pneumolysoids will elicit protection in humans. Antibodies to pneumolysin presumably impart protection by neutralizing the biological properties of the toxin rather than by stimulating opsonophagocytic clearance of the invading bacteria.

PspC

PspC (also called CbpA, SpSA, or Hic) is another pneumococcal surface protein (47–50). This virulence protein plays a role in adherence and colonization (47,51), and immunity to PspC can protect against colonization and invasive disease (49,51,52). PspC binds secretory IgA (48), factor H binding (50,53,54), and the polyimmunoglobulin receptor of the host (55). The binding of PspC to factor H results in inhibition of alternative pathway C3 activation (22). The multiple names for PspC came from the different activities of the protein and because of its variable mosaic protein, which includes alleles encoding very different domain structure (49,50,56). PspC was the first name given to this family of alleles in Gene Bank (09/26/1996).

The domain structure of many PspC molecules is reminiscent to that of PspA. The range of sizes of PspA and PspC overlap, and some PspC molecules have α-helical domains that are highly homologous to portions of the α-helical domain of some PspA molecules (49). The proline-rich domains of PspC are very similar to and frequently indistinguishable from those of PspA. The PspC of about 75% of pneumococci have a choline-binding domain indistinguishable from PspA (49,57). The remaining pneumococci produce a PspC called Hic in which the choline-binding region is replaced by an LPXTG motif associated with attachment of proteins to the cell wall peptide cross-bridge by the enzyme sortase (50,56). Eleven major groups of PspC proteins exist, and a nomenclature based on differences in the domain structures of the encoded proteins has been proposed (56).

Pneumococcal Surface Antigen A

PsaA is the metal-binding lipoprotein component of an ATP-binding cassette (ABC) transport system with specificity for Mn^{2+} (58). Defined $PsaA^-$ mutants of *S. pneumoniae* are virtually avirulent for mice and exhibit markedly reduced adherence in vitro to human type II pneumocytes (59). This is presumed to be a consequence of a requirement for Mn^{2+} as a cofactor or for regulation of expression of other virulence factors (e.g., adhesins), and/or growth retardation due to an inability to scavenge this metal in vivo (60,61). The avirulence of $PsaA^-$ pneumococci might also be due to the fact that they are highly susceptible to oxidative stress (62).

Although PsaA is less efficacious as a vaccine than pneumolysin or PspA, it confers partial protection against intraperitoneal challenge with *S. pneumoniae* (63). PsaA is only 7 nm at its longest axis (64), making it unlikely that when anchored to the outer face of the cell membrane via its N-terminal lipid moiety, it is well exposed on the outer surface of the pneumococcus. Thus, the observed protection against IP challenge is presumably due to in vivo blockade of ion transport, resulting from antibody that has diffused through the capsule and cell wall. Intranasal (IN) immunization with PsaA leads to efficient protection against colonization (6), but this may be due to the effects of CD4 cells (65), which would not require surface exposure of PsaA.

Autolysin

Autolysin, an *N*-acetylmuramoyl-*L*-alanine amidase, is a virulence factor (66,67) that is responsible for pneumococcal autolysis, following pneumococcal death. Isolated autolysin elicits protective immune responses (68). The observation that antibodies to autolysin and pneumolysin do not have synergistic protective effects suggests that the virulence mechanism of autolysin is the autolytic release of pneumolysin, which carries out the virulence functions (68). Mutations in either autolysin or pneumolysin in capsular type 2 strain D39 reduces virulence in a lung inoculation model (67).

Neuraminidase and Hyaluronidase

Streptococcus pneumoniae produces a large number of hydrolytic enzymes, some of which degrade host glycoproteins or extracellular matrix (69). A few of these are known virulence factors, including the neuraminidases NanA and NanB and the hyaluronate lyase (70–72). NanA is capable of cleaving terminal sialic acid from host glycoconjugates and in so doing, unmasks targets for pneumococcal adhesins (73). Some NanA mutant strains are more efficiently cleared from the nose and lung (74,75). Immunization with NanA extends the life of mice in an IN sepsis model and also protects against colonization and otitis media in chinchillas (70,76). Hyaluronate lyase is predicted to be a surface-bound protein in the pneumococcus. It degrades hyaluronic acid, a component of basement membranes and connective tissue; a *hyl* mutant exhibited reduced virulence in a mouse bacteremia model (71).

PhtA, B, D, and E

The *h*istidine *t*riad *p*roteins, designated PhtA, B, D, and E, are potential vaccine candidates (77). Their function is unknown but they are surface-exposed, and antibodies to PhtA and PhtD are found in human convalescent sera. Some Pht proteins elicit immunity against systemic challenge with pneumococci (77,78). However, in a direct comparative study, the most promising of these (PhtB and PhtE) were found to be less efficacious than either pneumolysoid or PspA (8).

PiuA and PiaA

The lipoproteins PiuA and PiaA are components of iron uptake transporters of *S. pneumoniae*. PiuA and PiaA are antigenically cross-reactive, and immunity to these proteins is reactive with pneumococci of nine different *S. pneumoniae* serotypes. Immunity to both PiuA and PiaA is more protective than immunity to either protein alone (79).

PotD

PotD is the surface transporter molecule of an ABC transporter for polyamine, an important nutrient of pneumococci. PotD appears to be highly conserved and is essential for virulence at least in sepsis and pneumonia models. Active immunity and passive antibody to PotD is protective against sepsis, pneumonia, and colonization in mice (80).