Review Article

Mycoplasma adhesion

SHMUEL RAZIN¹* and ENNO JACOBS²

¹Department of Membrane and Ultrastructure Research, The Hebrew University – Hadassah Medical School, Jerusalem, Israel 91010

²Institute for Medical Microbiology and Hygiene, University of Freiburg, D-7800 Freiburg, Germany

Introduction

The mycoplasmas (class *Mollicutes*) are widespread parasites of man, animals, plants and insects (Razin & Barile, 1985). They are distinguished from other prokaryotes by the total lack of a cell wall and by their minute dimensions. In fact, they can be considered as the smallest self-replicating organisms. Some mycoplasmas, like *M. genitalium*, have a genome of about 600 kb, estimated to comprise less than 500 genes (Razin, 1985a). These organisms are, therefore, attractive candidates for genome sequencing, an initial step already taken towards achieving the ambitious goal of complete definition in molecular terms of the machinery of a self-replicating organism (Morowitz, 1984).

The great majority of human and animal mycoplasmas colonize the epithelial linings of the respiratory and urogenital tracts, and can be considered as typical surface parasites. The lack of a cell wall and any of the appendages, like fimbriae, associated with adhesion of other prokaryotes, points to the fact that the mycoplasmal cell components responsible for attachment, termed adhesins, must be part of their cell membrane. Moreover, the lack of a cell wall facilitates direct contact of the mycoplasma membrane adhesins with the specific receptors on the host cell membrane, creating a condition which, in principle, could lead to fusion of the two membranes, and transfer or exchange of membrane components. While solid proof for occurrence of these events is still missing, it is clear that the close contact of the mycoplasma with the host cell membrane creates a micro-environment facilitating the accumulation of end-products of mycoplasma metabolism, like peroxide and superoxide radicals, thereby causing oxidative damage

to the host membranes (Razin, 1985b, 1986; Almagor *et al.*, 1986).

Adhesion of mycoplasmas to host cells is a prerequisite for colonization by the parasite and for infection. The loss of adhesion capacity by mutation results in loss of infectivity, and reversion to the cytoadhering phenotype is accompanied by regaining infectivity and virulence (Krause *et al.*, 1982, 1983). Most of the recent studies on mycoplasma adhesion have been focused on the human pathogens *M. pneumoniae* and *M. genitalium*. Some work has also been done on adhesion of another human pathogen, *Ureaplasma urealyticum* (Saada *et al.*, 1991; Robertson & Sherburne, 1991). These studies have not progressed far, due to technical difficulties resulting from the extremely poor yields of ureaplasmas cultivated *in vitro*. Hence, this review will essentially cover the significant progress made during the last 5 years or so on the molecular characterization of the *M. pneumoniae* and *M. genitalium* adhesins and the receptor sites of their hosts. For previous comprehensive reviews on mycoplasma adhesion the reader is referred to Razin (1985b, 1986) and Razin & Yogev (1988).

The organisms studied

M. pneumoniae is a well-established pathogen of the human respiratory tract, causing pneumonia (primary atypical pneumonia, 'walking pneumonia'). Children and young adults are the populations most at risk, whereas very young children (< 3 years) or adults (> 30 years) are less frequently affected (Cassell *et al.*, 1985; Jacobs, 1991). Several adverse events may contribute to the severity of the pneumonia. For example, exacerbation of the host cellular response on second contact with the parasite may aggravate the disease, promote a

* Author for correspondence. Tel. 2 428 008; fax 2 784 010.

more generalized inflammation and, by a still unknown mechanism, lead to post-infection complications such as exanthema and heart and central nervous system manifestations. In addition, *M. pneumoniae* infection may induce auto-antibodies, predominantly of the IgM class, including cold agglutinins to erythrocytes, and antibodies reacting with brain, lung, smooth muscle, mitotic spindle apparatus etc.; these antibodies, apparently, are responsible for the variety of post-infection sequelae affecting the central nervous system, heart, pancreas, joints, blood etc. (for references see Jacobs, 1991).

M. pneumoniae exhibits the typical polymorphism of mycoplasmas, but most prominent are flask-shaped elongated organisms showing a tiny tip at one of the poles. This differentiated organelle, termed the tip organelle, tip structure or terminal structure, functions as an attachment organelle. As can be seen in Fig. 1, the tip organelle consists of an internal rod surrounded by a lucent space enclosed by the cell membrane. Meng & Pfister (1980) were the first to show that partial removal of the cell membrane by brief treatment with 1% Triton X-100 exposes the striated rod of the tip organelle, and also reveals thin fibrils associated with it. These observations, confirmed and extended by Göbel *et al.* (1981) and by Kahane *et al.* (1985) were interpreted as the presence of a primitive cytoskeleton (also termed the Triton shell) responsible for contractibility and motility of this organism. Motility of *M. pneumoniae* is of a peculiar type: the organism glides on a solid surface with the tip organelle directed towards the surface in the direction of movement (Bredt, 1979). While the mechanism of mycoplasma motility remains unclear, it is reasonable to assume that motility plays a role in the penetration of *M. pneumoniae* through the mucus layer covering the tracheal ciliary epithelium, with the tip organelle leading the way to reach its destination – the membrane of the epithelial cell at the base of the cilia.

M. genitalium shares several important properties with *M. pneumoniae*: a flask-shaped morphology with a tip organelle, and gliding motility. Immunoblots show marked serological cross-reactivity between *M. genitalium* and *M. pneumoniae* cell proteins, supported by the significant sharing of genomic sequences between the two mycoplasmas (Yogev & Razin, 1986). Yet, despite the marked relatedness to *M. pneumoniae*, there is no doubt that *M. genitalium* deserves the status of a separate species. This is based on the significant difference in genomic DNA content [about 33 mol% guanine + cytosine (G + C) in *M. genitalium*, compared to 40 mol% in *M. pneumoniae*], DNA homology values between the two mycoplasmas lower than 8% (Yogev & Razin, 1986), totally different genomic cleavage patterns of the two organisms (Yogev *et al.*, 1988) and a marked

difference in genome size. While the values reported for the *M. pneumoniae* genome are in the 800 kb range (Wenzel & Herrmann, 1989; Krause & Mawn, 1990; Su & Baseman, 1990) the genome of *M. genitalium* is less than 600 kb in size (Su & Baseman, 1990; Colman *et al.*, 1990b), the smallest genome recorded for any mycoplasma and, in fact, for a self-replicating organism. Whether this extremely small genome reflects the exceptionally fastidious nature of *M. genitalium* is not known but is plausible. Nevertheless, despite all of the above differences, *M. genitalium* resembles *M. pneumoniae* very closely in the nature of the cell components responsible for adherence to host cells, as will be described in detail in the following sections.

Little is known about the pathogenicity of *M. genitalium* for man. Originally isolated by Tully *et al.* (1981) from the urethral discharge of two men suffering from non-gonococcal urethritis, this mycoplasma has been considered a possible agent of non-gonococcal urethritis (Taylor-Robinson *et al.*, 1985a). However, being extremely difficult to cultivate from clinical material, evidence for the role of *M. genitalium* in urogenital infections is still largely indirect, based on serological tests and more recently on the detection by gene probes of the organism's DNA in clinical specimens (Jensen *et al.*, 1991), and on experimental infections in primates (Taylor-Robinson *et al.*, 1985b; Tully & Baseman, 1991). Complicating the issue is the finding of *M. genitalium* together with *M. pneumoniae* in the respiratory tract of mycoplasmal pneumonia patients (Baseman *et al.*, 1988) and, more recently, in the synovial fluid of a patient with polyarthritis after primary pneumonia (Tully & Baseman, 1991), indicating that *M. genitalium* may colonize the respiratory tract and possibly act there as a pathogen by itself or in association with *M. pneumoniae*.

The P1 operon, genes and proteins

The first indication of the protein nature of *M. pneumoniae* adhesins came from observations that mild trypsin treatment of *M. pneumoniae* cells abolished their ability to adhere to tracheal epithelium and erythrocytes (Hu *et al.*, 1977; Gorski & Bredt, 1977). Electrophoretic analysis of the treated mycoplasmas indicated the digestion of two membrane proteins. The protein with the higher molecular mass, estimated at 170–190 kDa, was named P1 (Hu *et al.*, 1977). Subsequent studies established that protein P1 is the major adhesin of *M. pneumoniae* (Hu *et al.*, 1982; Baseman *et al.*, 1982; Feldner *et al.*, 1982). *M. pneumoniae* organisms that lack P1, or are unable to densely cluster or properly position it at the surface of the tip organelle, fail to attach to the

Fig. 1. Thin section of *M. pneumoniae* infecting a tracheal ring organ culture. The filamentous mycoplasmas (M) show the tip organelle adhering to a non-ciliated epithelial cell. The tip organelle (T) exhibits the rod structure surrounded by a lucent space enclosed by the mycoplasma membrane. Bar, 0.1 μ m. (From Wilson & Collier, 1976; reproduced with the permission of the American Society for Microbiology.)

Table 1. *Molecular properties of mycoplasma adhesins*Data from Su *et al.* (1987), Inamine *et al.* (1988a, b, 1989), Dallo *et al.* (1989a, 1990a) and Mader *et al.* (1991).

	P1 adhesin (<i>M. pneumoniae</i>)	MgPa adhesin (<i>M. genitalium</i>)	P30 adhesin (<i>M. pneumoniae</i>)
<i>Gene</i>			
No. of nucleotides	4881	4335	825
Mol% G + C	53.5	39.9	54.4
Tryptophan codons	TGA(21) and TGG(16)	TGA(16) and TGG(12)	TGA(1) and TGG(1)
Organization in genome	Part of a three-gene operon (ORF-4-P1-ORF-6)	Part of a three-gene operon (ORF-1-MgPa-ORF-3)	Apparently part of an operon
<i>Protein</i>			
<i>(a) Precursor</i>			
No. of amino acids	1627	1445	275
Molecular mass (Da)	176288	159660	29473
Leader sequence	59 amino acids	58 amino acids	Not defined
<i>(b) Mature protein</i>			
Molecular mass (Da)	169758	153134	Not defined
Cysteine	Absent	Absent	One residue
Proline	Proline-rich C-terminus	Proline-rich C-terminus	Proline-rich C-terminus
Sensitivity to trypsin	Sensitive	Resistant	Resistant
Serological activity	Immunodominant	Immunodominant	Immunodominant
Localization in mycoplasma	Clustered at tip organelle	Clustered at tip organelle	Clustered at tip organelle

respiratory epithelium and consequently are avirulent (Baseman *et al.*, 1982; Krause *et al.*, 1982). Much of the more recent work on *M. pneumoniae* adhesion has centred therefore on the molecular characteristics of the P1 adhesin, making use of new molecular genetics methodology.

The P1 gene was cloned and sequenced independently by Su *et al.* (1987) and by Inamine *et al.* (1988a) with essentially identical results, an outstanding feat considering the size of the gene (almost 5000 nucleotides long). Additionally, Inamine *et al.* (1988a, b) showed the P1 gene to be part of a three-gene operon (Table 1).

Elucidation of the nucleotide sequence of the P1 gene revealed some peculiar properties concerning the structural gene itself and the deduced amino acid composition of the encoded P1 protein. As can be seen in Table 1 the G + C content of the P1 gene is 53.5 mol%, considerably higher than the 40 mol% of the entire *M. pneumoniae* genome. Together with many other mycoplasmas, *M. pneumoniae* uses the universal stop codon UGA as a tryptophan codon (Yamao *et al.*, 1985; Blanchard, 1990). Thus, the P1 gene carries more UGA codons for tryptophan than UGG, the conventional tryptophan codon (Table 1). A tRNA recognizing UGA as tryptophan has, in fact, been detected in *M. pneumoniae* (Inamine *et al.*, 1989). As to the P1 protein, being a surface-exposed membrane protein it undergoes processing. The leader or signal sequence, cleaved and removed on protein maturation, is rather long (Table 1), resembling some mitochondrial and chloroplast signal peptides and differing from bacterial signal peptides which

are usually 20 to 40 amino acids long (Inamine *et al.*, 1988a). The large P1 protein is completely devoid of cysteine, deduced from the P1 gene analysis and confirmed by lack of [³⁵S]cysteine incorporation into the P1 protein during growth (Inamine *et al.*, 1988a). Consequently, the P1 protein is devoid of intramolecular disulphide bonding, conferring considerable flexibility to its long polypeptide chain. Another peculiar property of the P1 protein is its high proline content, particularly at the C-terminus region associated with cytoadherence (Dallo *et al.*, 1988). This is rather unusual and may place structural restraint on the protein and thus assist in topological organization of the adhesin in the membrane (see below – Conformation of adhesins in membranes). Interestingly, other adhesins of *M. pneumoniae* and *M. genitalium* also have a high proline content (Table 1).

The P1 operon comprises three open-reading frames: ORF-4, P1 and ORF-6. Nucleotide sequencing indicates the absence of cysteine in ORF-6, resembling in this respect P1, while ORF-4 contains three cysteine residues (Inamine *et al.*, 1988b). According to its size, ORF-4 should encode a 28 kDa protein, P1 a 170 kDa protein and ORF-6 a 130 kDa protein. Since genes contained within an operon are often involved in related functions, one could predict that the ORF-4 and ORF-6 proteins may participate in *M. pneumoniae* adhesion. In order to characterize the proteins translated from ORF-4 and ORF-6, Sperker *et al.* (1991) constructed gene fusions between the N-terminus of the RNA replicase of the *E. coli* bacteriophage MS2 and selected regions of ORF-4 and ORF-6. The fusion proteins synthesized in *E. coli*

were used to immunize mice. Unfortunately, for some unknown reason, the antisera directed against ORF-4 sequences did not recognize *M. pneumoniae* antigens in immunoblots. The results obtained with the ORF-6 sequences were also unexpected: instead of a single 130 kDa protein, two *M. pneumoniae* proteins, one of 40 kDa and the other of 90 kDa, were recognized by the specific antisera. The 40 kDa protein represented the N-terminus, and the 90 kDa protein the C-terminal part of ORF-6. The simplest explanation for this finding would be that the two proteins arise from a common 130 kDa precursor by proteolytic cleavage. Yet, such a precursor was not identified in the immunoblots, even in *M. pneumoniae* cells from young cultures (Sperker *et al.*, 1991).

Both the 40 kDa and 90 kDa proteins were missing from the non-cytadsorbing avirulent B176 *M. pneumoniae* strain, lending support to the notion that these proteins may, indeed, be involved in adherence. Support for this idea comes also from the observation of Vu *et al.* (1987) that all convalescent-phase human sera reacted in immunoblots with a 90 kDa protein in all *M. pneumoniae* strains, except for B176. Obviously, if the avirulent B176 strain could be converted into a pathogenic adhering strain by transformation with ORF-6 DNA, proof for the role of the 90 kDa protein in adherence would be achieved. Until then, one can only hypothesize about its role, and more generally about the role of the proteins encoded by ORF-4 and ORF-6 of the P1 operon.

The P30 adhesin

Although P1 is regarded as the major *M. pneumoniae* adhesin, it is by no means the only adhesin in this mycoplasma. Baseman *et al.* (1987) showed that a mAb which blocked *M. pneumoniae* attachment to chicken erythrocytes was directed to a 32 kDa protein present in wild-type *M. pneumoniae* but absent from class II non-adhering avirulent mutants (see below – Accessory proteins). Resembling P1, this protein is clustered at the tip organelle, and is highly immunogenic so that antibodies to it can be detected in convalescent-phase human sera, and in sera of hamsters infected with *M. pneumoniae* (Leith *et al.*, 1983; Vu *et al.*, 1987). Recognition of this protein as an adhesin led Dallo *et al.* (1990a) to clone its gene and characterize it. As can be seen in Table 1, the protein, designated P30, shares a number of properties with P1, notably a proline-rich C-terminus, expressed also by substantial amino acid sequence homology with the C-terminus of P1. In addition, the C-terminus of P30 contains three types of repeat sequences, each consisting of six amino acids. The function of these repeat sequences remains unknown.

Repeated P1 gene sequences and sequence divergence

The occurrence of multiple copies of partial gene sequences has been reported for many pathogenic prokaryotes and eukaryotes, providing genetic rearrangement mechanisms for inducing variation in surface antigens and adhesins. These variations facilitate evasion by the parasite of the host defence mechanisms (Yogev *et al.*, 1991). The P1 gene, being the gene for the major *M. pneumoniae* adhesin, presented itself as an obvious candidate for investigating genetic rearrangements. Su *et al.* (1988) employed 14 subclones spanning the entire P1 gene sequence as probes in Southern blot hybridizations with genomic *M. pneumoniae* DNA. Approximately one-third of the P1 structural gene was present as a single copy, while the other two-thirds of the gene sequences were present as multiple copies. Hence, there is only one complete copy of the P1 gene in the *M. pneumoniae* genome, but sequences of this gene are distributed in other regions of the genome. Wenzel & Herrmann (1988a, b), working on physical mapping of the *M. pneumoniae* genome, also encountered the presence of repetitive DNA sequences dispersed on the genome, some of which were identified as P1 gene sequences. The picture became more complex with the finding of another type of repetitive element, this time consisting of sequences of the ORF-6 gene of the P1 operon (Colman *et al.*, 1990a). To clarify the issue, a comprehensive analysis of all known repeated DNA elements, as to size, copy number, distribution on the genome and conservation within strains, was carried out by Ruland *et al.* (1990) making use of the complete and ordered *M. pneumoniae* genomic library constructed by them.

The conclusions that can be drawn from the data are as follows. (i) No significant distribution patterns of the repeat elements can be seen on the genome, but a certain accumulation of the elements was observed around the P1 operon. (ii) The total size of repeats is about 50 kb, that is about 6% of the genome. As mentioned above, detection of amplified DNA sequences in a pathogenic mycoplasma is not exceptional and is not surprising by itself. However, most surprising is the large size and relatively high copy number of these elements, present in a genome considered among the smallest of self-replicating organisms. (iii) It is hard to believe that the repeat elements, comprising 6% of the genome, do not play an important role in the life of the pathogen. Su *et al.* (1988) put forward several suggestions for possible functions of the repeat sequences. Accordingly, the large P1 gene consists of a mosaic of DNA segments which code for different functions. Some sequences, associated with cytoadherence, may be highly specific and

conserved, while others may code for more common structural-functional domains such as transmembrane domains, signal sequences etc. Gene recombination among these segments may occur. So far there is no evidence for transcription or translation of the repeating elements, neither is there any real indication for their function.

Cell protein profiles, immunoblots and DNA restriction patterns have demonstrated the marked genotypic homogeneity of the species *M. pneumoniae*, in marked contrast with most other species of mollicutes (Chandler *et al.*, 1982; Vu *et al.*, 1987; Yogev *et al.*, 1988). Is this genotypic homogeneity reflected in the structure of the P1 gene? This is an important and legitimate question in the light of the high frequency of appearance of non-adhering *M. pneumoniae* mutants (see below – Accessory proteins), and the presence of multiple copies of P1 gene sequences, which would be expected to facilitate genetic recombination and variation. Examination of 25 clinical isolates of *M. pneumoniae* revealed no detectable differences in the cell protein profiles, including the P1 protein. Furthermore, the P1 protein of all the different isolates reacted with anti-P1 mAbs generated against the wild-type strain M129-B16 (Dallo *et al.*, 1990*b*; Su *et al.*, 1990*b*). The existence of some variations in the P1 gene could be detected only by Southern blot analysis with the entire battery of non-overlapping subclones of the P1 gene, and by P1 gene sequencing. The 25 isolates could, accordingly, be split into two groups: group I consisting of three isolates and strain M129-B16, and group II consisting of all other isolates. Within each of the groups the P1 nucleotide sequences were identical or different in only one nucleotide. The difference was more discernible between the two groups. Protein P1 of group I consisted of 1627 amino acids while that of group II consisted of 1635 amino acids. The nucleotide variations between the groups were located mostly in the multiple copy regions of the P1 genes. Su *et al.* (1990*a*) expressed surprise that despite expectations, the P1 gene revealed such a highly conserved nature. However, considering the crucial role of the P1 adhesin in *M. pneumoniae* parasitism, one should not be surprised at the stability of the P1 gene. Minor sequence variations are known to occur in the highly conserved ribosomal RNA and elongation factor genes of mycoplasmas without affecting the function of their encoded products (Razin, 1985*a*; Yogev *et al.*, 1988, 1990) so that minor variations in the P1 gene could be expected. The question is really whether these minor variations have any effect on virulence and on the immune response of the host, as proposed by Su *et al.* (1990*a, b*). No answer to this question can be given at present. As will be discussed below, known reasons for loss of adherence in *M. pneumoniae* are associated with loss of accessory proteins, energized state of cells and

apparent changes in membrane distribution and exposure of the P1 adhesin, rather than with changes in the P1 molecule itself.

Accessory proteins

The spontaneous appearance of non-adhering mutants of *M. pneumoniae* occurs at a high frequency (Krause *et al.*, 1982, 1983) and cells with a non-adherence phenotype typically comprise more than 50% of the population of virulent *M. pneumoniae* cultures (Kahane *et al.*, 1985). The finding that P1 is usually not lost from non-adhering cells indicates that P1 alone is not sufficient for mediating cytoadherence. In fact, data accumulated in the last decade show that *M. pneumoniae* cytoadherence is a complex, multifactorial process, involving, in addition to the adhesins P1 and P30, other membrane proteins and cytoskeletal elements. Furthermore, for the complex to function metabolic energy is needed, apparently for the concentration and proper alignment of the adhesins at the tip organelle in a conformation assuring their tight binding to host cell receptors.

The cytoadherence-negative mutants have been grouped into four classes (Krause *et al.*, 1982; Stevens & Krause, 1991). Class I consists of mutants lacking five high-molecular-mass proteins designated HMW1 to HMW5, ranging in molecular mass from 140 to over 340 kDa (Stevens & Krause, 1990). Class II mutants lack the P30 adhesin. Class III mutants lack proteins designated A, B and C (72, 85 and 37 kDa, respectively), while class IV, represented by a single mutant, lacks protein P1 in addition to proteins A, B and C. In all cases, the re-acquisition of the missing protein(s) results in reversion to the adherence-positive phenotype (Krause *et al.*, 1983). The HMW and A, B, and C proteins were termed accessory proteins as they could not be defined as adhesins *per se*, but are required for the proper functioning of the adhesins.

Some progress has recently been made in elucidating the nature of the defects leading to loss of cytoadherence by some of the mutants, and initial steps were taken to characterize the molecular properties of the accessory proteins. A problem recently solved concerns the absence of P1 from the class IV mutant. The defect was shown to be caused by a frameshift mutation due to an extra adenine in a stretch of normally seven adenines near the N-terminal region of the P1 gene. This frameshift mutation, caused probably by DNA polymerase slippage at the reiterated bases during DNA replication, results in early termination of protein translation (Su *et al.*, 1989). The molecular characterization of the accessory proteins has been focused on the HMW proteins. To the four HMW proteins absent from

class I mutants (Krause *et al.*, 1982), a fifth one, HMW5, was recently added (Stevens & Krause, 1990). This disulphide-linked protein (> 340 kDa) consists apparently of two 190 kDa subunits, probably identical with HMW2. As all the HMW proteins are lost or re-acquired simultaneously, their expression must be co-ordinately regulated (Krause *et al.*, 1983). Efforts have recently been made by D. C. Krause and his associates to clone and characterize the genes of the HMW proteins. Results are still fragmentary and incomplete so that definite conclusions as to the structure of the genes cannot be drawn, but it appears that the genes for HMW1 and HMW3 are adjacent to each other on the *M. pneumoniae* genome, in line with their presumed co-ordinate expression (Ogle *et al.*, 1991; Krause & Lee, 1991).

How do the accessory proteins promote adherence? As already mentioned, clustering of P1 and P30 at the tip organelle appears vital to attachment, as clustering provides a critical concentration of adhesin molecules required for securing a stable primary association with receptor molecules on the host cells. In the non-adhering mutants P1 fails to cluster at the tip organelle (Baseman *et al.*, 1982b). Hence one can speculate that one or more of the accessory proteins plays a role in the lateral movement and concentration of the adhesin molecules at the tip organelle. How this is accomplished is still a matter for speculation. A plausible hypothesis is that to fulfil this role the accessory proteins should be associated with the mycoplasma cytoskeleton, which is responsible not only for P1 lateral movement and proper orientation, but also for changes in cell shape, cell division and motility (Razin, 1986). In support of this notion Stevens & Krause (1991) have shown that the HMW proteins are apparently part of the cytoskeleton-like Triton shell of *M. pneumoniae* (see previous section – The organisms studied) and are localized primarily in the filamentous extensions of the mycoplasma cells, in proximity to the tip organelle. It could further be proposed that the P1 molecule is associated, through its C-terminus, with the cytoskeleton, an association mediated by one or more accessory proteins (Kahane *et al.*, 1985).

Energy is needed for the cytoskeleton to function. A 55 kDa phosphoprotein recently detected in motile *Mycoplasma* and *Spiroplasma* species, including *M. pneumoniae* and *M. gallisepticum*, may well be an important constituent of the cytoskeleton (Platt & Rottem, 1990). Phosphorylation of the protein requires ATP and thus depends on active metabolism. It has long been known that for *M. pneumoniae* to adhere an energy source is needed and adherence does not take place at 4 °C (Feldner *et al.*, 1981), findings which were recently confirmed by Krivan *et al.* (1989) who showed that only metabolically active *M. pneumoniae* cells bind to the sulphated receptors (see below – Receptors).

The MgPa operon, genes and proteins

M. genitalium carries an adhesin which resembles very much the *M. pneumoniae* adhesin P1. The similarities are expressed at all levels: the structural gene, encoded protein, immunogenicity, clustering at the tip organelle etc. (Table 1). In short, the major *M. genitalium* adhesin, termed MgPa (Hu *et al.*, 1987) can be regarded as the counterpart or analogue of P1. Like P1, the MgPa structural gene is the central gene in a three-gene operon consisting of ORF-1 (29 kDa protein), ORF-2 (160 kDa protein, MgPa) and ORF-3 (114 kDa protein). The deduced amino acid sequences of the proteins show extensive homologies with those of the homologous sequences of the P1 operon (Inamine *et al.*, 1989; Dallo *et al.*, 1989a, b). As with P1, the MgPa gene has more UGA than UGG codons for tryptophan, is devoid of cysteine, and has a proline-rich C-terminus (Table 1). Approximately half of the MgPa gene sequences exist as multiple copies dispersed on the *M. genitalium* chromosome, again resembling findings with P1 (Dallo & Baseman, 1991).

Most significantly, MgPa, although smaller than P1, exhibits significant regions of amino acid sequence homology with the latter. Thus 85% of the deduced amino acid sequence of MgPa exhibits 42% to 74% identity with regions of P1, and hydrophobicity plots of the amino acid C-termini of the two proteins are almost superimposable (Dallo *et al.*, 1989a). Computer matching of the deduced amino acid sequences of MgPa and P1 (Mader *et al.* 1991), using gaps and loops to achieve maximum homology, has revealed that 747 amino acids on the entire sequences of MgPa and P1 are identical, and five of the seven hydrophobic regions of the proteins are located in corresponding regions (Fig. 2).

In the light of this remarkable sequence homology, demonstration of epitopes common to both MgPa and P1 could be expected. Epitope analysis, employing overlapping octapeptides of a P1 region immunologically active in human *M. pneumoniae* infections, and of the corresponding MgPa regions, has revealed six common epitopes, but also one *M. genitalium*-specific and two *M. pneumoniae*-specific epitopes in sera of guinea pigs infected intranasally with *M. genitalium* or *M. pneumoniae*, respectively (Jacobs *et al.*, 1991). Moreover, mAbs generated by Morrison-Plummer *et al.* (1987) against MgPa inhibited adhesion of *M. pneumoniae*, reinforcing the chemical and functional relatedness of the two adhesins. It is possible that the marked sequence homology between MgPa and P1 is also shared by the other proteins encoded by the operons MgPa and P1. An indication that this may be so is the finding that an antibody to a protein(s) encoded by ORF-6 of the P1 operon cross-reacted with a *M. genitalium* protein of

118 kDa, the size expected for ORF-3, the open-reading frame in the MgPa operon corresponding in position to ORF-6 (Sperker *et al.*, 1991). Repetitive DNA sequences of MgPa origin (see above) appear also to be present in the *M. genitalium* genome (Dallo & Baseman, 1991). An MgPa probe hybridized not only with the *M. genitalium* DNA fragment carrying the MgPa gene, but also, though significantly less effectively, with a number of other genomic DNA fragments (Colman *et al.*, 1990a).

The striking similarities between the MgPa and P1 adhesins led Dallo *et al.* (1989a, b) to suggest that the two proteins may be the product of an ancestral gene that underwent a horizontal gene transfer event. They went further to propose that the apparently conserved regions in the two adhesins provide an explanation for the finding of both *M. genitalium* and *M. pneumoniae* in the human respiratory tract (Baseman *et al.*, 1988), assuming that tissue tropism may be the consequence of similar receptor-dependent interactions mediated by these two adhesins.

Conformation of adhesins in membranes

Having at hand the complete amino acid sequence of the P1 protein enabled a computerized analysis of the sequence to be made in order to predict the molecular topography of P1. Since all computer predicting methods are based on parameters such as hydrophilicity, hydrophobicity and flexibility of protein segments, the topographical information gained should be considered as indicative rather than definitive, and is limited to a two-dimensional profile of the protein. Even if, for example, all membrane-spanning segments of the P1 protein are identified by the computer method (Figs 2 and 3), the structure of regions exposed on the cell surface or facing the cytoplasm has still to be determined. Therefore, the computer predictions of the P1 protein structure helped to provide the first clues for probable antigenic regions (based on hydrophilic or flexible segments) but could not replace the need for classical experimental approaches to provide a more definite determination of the P1 protein topography.

One of the established computer prediction methods to define the topology of membrane-spanning segments involves determining hydrophobic amino acid sequences with a favourable alpha helix structure. Using the hydrophobic moment plot of Eisenberg *et al.* (1984), six hydrophobic membrane-embedded segments of the mature P1 protein (M1 to M6) were calculated in addition to an hydrophobic segment in the signal peptide sequence, which is cleaved and eliminated during the P1 maturation process (Fig. 3). Interestingly, comparison of the topographical features of the P1 protein with those of

the MgPa protein of *M. genitalium* reveals that the two resemble each other in the location of the hydrophobic segments M1 and M2 (Fig. 2). The most hydrophobic segments predicted in both P1 and MgPa are found in the C-terminal region, indicating the strong association of this region with the membrane. On the other hand, the hydrophilic and flexible segments (Hopp & Woods, 1981; Karplus & Schulz, 1985) are often found to correlate with extracellular and antigenic sites. Running the computer programs for the P1 amino acid sequence revealed that all hydrophilic and flexible regions are located in front or following the M1–M2 hydrophobic area, indicating that most of the P1 protein might be exposed on the external cell surface, whereas the almost uncharged, proline-rich and inflexible C-terminus is apparently facing the cytoplasmic compartment (see Fig. 4).

To evaluate and supplement the computer based data, peptides (15 to 20 amino acids long) of the computer-predicted hydrophilic and flexible P1 regions, a peptide of the N-terminus and two peptides of the C-terminus (Fig. 3) were synthesized and used to produce monoclonal and polyclonal antisera in mice. By immunofluorescence all the antisera, except for those directed against the C-terminus peptides, gave a positive signal with freshly harvested, live *M. pneumoniae* cells. In addition, proteolysis experiments with carboxypeptidase Y showed the C-terminus to be cleaved only when present in inside-out vesicles but not in live mycoplasma cells (E. Jacobs, unpublished data). Both approaches thus support the computer predictions that the C-terminus of P1 is facing the cytoplasm.

Anti-P1 antibodies are not only useful for topographic studies, but can also be applied to epitope mapping of distinct functions. For this purpose anti-P1 mAbs which inhibit *M. pneumoniae* adherence to hamster epithelial cells and to erythrocytes were produced (Dallo *et al.*, 1988; Jacobs *et al.*, 1989; Gerstenecker & Jacobs, 1990). The P1 protein was isolated and purified from *M. pneumoniae* cells (Jacobs *et al.*, 1988b), and chemically cleaved by cyanogen bromide. The cleaved fragments were separated by two-dimensional PAGE and transferred to nitrocellulose. The separated peptide spots were tested for mAb binding. Peptides showing a positive immunoblot reaction with the adherence-inhibiting mAbs were cut out of the nitrocellulose membrane to sequence their N-terminal end by Edman degradation (Jacobs *et al.*, 1989). The results of these experiments revealed at least two regions of the P1 protein reacting with the cytoadherence-inhibiting mAbs (the N-region and the D1-region in Fig. 3). In addition, a third region (D2-region), positioned ahead of the hydrophobic M6-region, was found by Dallo *et al.* (1988) to react with adherence-inhibiting mAbs. More detailed studies, using

(a) MgPa protein of *M. genitalium* (b) P1 protein of *M. pneumoniae*

Fig. 2. Common structural elements of the MgPa protein of *M. genitalium* (a) and the P1 protein of *M. pneumoniae* (b). The scheme shows the large signal peptides of 58 and 59 amino acids, respectively, cleaved during the maturation process, and the six predicted hydrophobic segments (M1 to M6) of which segments M1 and M2 are located at almost the same position in both proteins. The most hydrophobic segment M6 is positioned in both proteins within the C-terminal region.

Fig. 3. Topological characteristics of the P1 protein of *M. pneumoniae*. The following are shown. 1. Binding sites of adherence-inhibiting monoclonal antibodies (mAb) within the N-terminal, the D1- and the D2-regions of the P1 protein. 2. Epitopes (human-Ab: a to m), i.e. the binding sites of human IgG serum antibodies of patients suffering from *M. pneumoniae* disease. 3. Computer predicted positions of regions with hydrophilicities (maximal in locations designated p1 to p3), according to Hopp & Woods (1981), with flexibilities (maximal in locations designated f1 to f10) according to Karplus & Schulz (1985), and β -turns calculated according to Chou & Fasman (1979). The areas designated M1 to M6 indicate predicted intramembranous segments, according to Eisenberg *et al.* (1984). [Modified from Jacobs, 1991; reproduced with the permission of Churchill Livingstone (Longman Group UK)].

overlapping synthetic octapeptides of different regions of the P1 protein, defined the mAb-binding sites in a more refined manner (Gerstenecker & Jacobs, 1990). The epitope-mapping experiments have led to the suggestion that the N-terminal region, the D1-domain and the D2-domain are arranged in such a close manner to enable the three different loops to fold under *in vivo* conditions and form a 'triangular-complex' which would exhibit stronger adherence capacity due to co-operative binding to the receptor molecule (Gerstenecker & Jacobs, 1990). Fig. 4 presents a model illustrating the

above hypothesis (Jacobs, 1991). This P1 model implies an adhesin receptor interaction which is different from that of fimbrial *E. coli* adhesin proteins in which the adhesin binding sites consist of rather rigid sequential amino acid sequences (Hacker, 1990). We speculate that the P1 adhesin complex is not rigid. During attachment to epithelial cells or to erythrocytes the three flexible P1 protein loops may arrange themselves around biochemically different receptor molecules (see below - Receptors) like a glove adapting to the hand. Electrostatic or hydrophobic interactions may be the leading forces of

Fig. 4. Cartoon representation of the P1 adhesin of *M. pneumoniae* showing the proposed adhesion site complex formed by the three extracellular loops (N-, D1- and D2-regions) of the P1 molecule, bound to the membrane through helices M1, M2 and M6. The position of the segments with lower hydrophobicity, M3, M4 and M5 is still unclear. The hydrophobic segment M6 crosses the cell membrane (CM), so that the C-terminus is located in the cytoplasm (CY) of the *M. pneumoniae* cell. [Modified from Jacobs, 1991; reproduced with the permission of Churchill Livingstone (Longman Group UK)].

this adhesin/receptor contact (Bredt *et al.*, 1983; Razin, 1985b).

Mycoplasma gallisepticum adhesins

There was extensive research on adhesion of the avian respiratory pathogen *M. gallisepticum* in the 1970s and early 1980s (Razin, 1985b, 1986) but the emphasis is now on studying adhesion of the human pathogenic mycoplasmas. Nevertheless, the availability of cloned genes and specific antibodies to the *M. pneumoniae* and *M. genitalium* adhesins prompted Dallo & Baseman (1990) to apply these reagents to *M. gallisepticum* cells, found previously to share with the two human mycoplasmas protein epitopes (Clyde & Hu, 1986), genomic sequences (Yogev & Razin, 1986) and sialoglycoconjugate receptors for adhesion (Baseman *et al.*, 1982a; Loomes *et al.*, 1984). The antiserum to P1 reacted in immunoblots with a 155 kDa protein of *M. gallisepticum*, and the cloned P1 and MgPa genes hybridized in Southern blots with genomic *M. gallisepticum* DNA, indicating the presence in *M. gallisepticum* of nucleotide sequences homologous to those of P1 and MgPa. These findings led Dallo & Baseman (1990) to propose that the three phylogenetically related mycoplasmas share a family of adhesin-

related genes. However, until the presumed 155 kDa adhesin of *M. gallisepticum* is characterized further, no definite conclusion can be drawn as to the validity of the above suggestion. Certainly, the presumed *M. gallisepticum* adhesin appears to differ significantly in its molecular properties from P1 and MgPa, as cytoadherence-inhibiting mAbs to P1 and MgPa do not react with the *M. gallisepticum* 155 kDa protein, and the subclone of the P1 gene that encodes the 13 amino acid sequence mediating cytoadherence (Dallo *et al.*, 1988) does not hybridize with *M. gallisepticum* DNA, even under low stringency conditions.

Receptors

The role of sialoglycoconjugates of host cell surfaces as receptors for *M. pneumoniae*, *M. genitalium*, *M. gallisepticum* and *M. synoviae* has long been established (for references see Razin, 1985b, 1986). Loomes *et al.* (1984, 1985) showed that *M. pneumoniae* receptors on human erythrocytes were glycoproteins and glycolipids with carbohydrate structures consisting of α 2-3-sialylated poly(*N*-acetylactosamine) sequences. More recent work by Roberts *et al.* (1989) has confirmed the role of sialylated glycoproteins as receptors for *M. pneumoniae*, defining the active proteins as those carrying terminal NeuAc(α 2-3)Gal(β 1-4)GlcNAc sequences.

Nevertheless, neuraminidase treatment has frequently failed to abolish completely, or even partially, the ability of various eukaryotic cells to bind *M. pneumoniae* (Bredt *et al.*, 1982; Geary & Gabridge, 1987), prompting a search for additional receptor molecules. This search, based on measuring binding of labelled *M. pneumoniae* cells to purified glycolipids or glycoproteins adsorbed to a solid surface, has indicated that sulphated glycolipids containing terminal Gal(3SO₄) β 1-residues function as receptors; obviously, these are insensitive to neuraminidase (Krivan *et al.*, 1989). Moreover, a sialic-acid-free glycoprotein, isolated from cultured human lung fibroblasts, has also been shown to serve as a receptor for *M. pneumoniae* (Geary *et al.*, 1990).

Clearly, there is more than one type of receptor for *M. pneumoniae*, and apparently for other adhering mycoplasmas as well. This should not come as a surprise considering that each mycoplasma may carry more than one adhesin type. Thus *M. pneumoniae* has at least two adhesins, P1 and P30 (Table 1). This notion finds support in the finding of Geary *et al.* (1990) that the glycoprotein receptor isolated from lung fibroblasts binds specifically to a 32 kDa protein of *M. pneumoniae*, a 90 kDa protein of *M. genitalium* and a 139 kDa protein of *M. gallisepticum*. Hence the major adhesins of *M. pneumoniae* and *M. genitalium*, P1 and MgPa, do not

seem to recognize this glycoprotein 'receptor'. It could be argued that this glycoprotein recognizes the 30 kDa (P30) adhesin of *M. pneumoniae* (Table 1). A possible way to examine this proposal would be to test purified adhesins for their binding to prospective receptor molecules, utilizing the methodology of Krivan *et al.* (1989) and Roberts *et al.* (1989).

Identification of the sialo-oligosaccharide type of receptors for *M. pneumoniae* enabled Loveless & Feizi (1989) to apply sequence-specific mAbs and lectins to demonstrate *in situ* the receptor structures for *M. pneumoniae* at the primary site of infection, the human bronchial epithelium. While the apical microvillar border and the cilia carried the receptors, the secretory cells and mucus lacked them, favouring attachment of the pathogen to the ciliated cells and avoiding the secreted mucus barrier.

Immunogenicity of adhesins and vaccine engineering

In order to test the idea of using P1 or MgPa as vaccinogens, ways have to be devised to prepare these adhesins in large quantities. Being hydrophobic integral membrane proteins hampers their isolation and purification, and in addition one has to take into account the high cost and great difficulties of cultivating *M. pneumoniae* and, more so, *M. genitalium*. Purified P1 has been isolated from *M. pneumoniae* cells either by immunoaffinity chromatography followed by SDS-PAGE and elution (Su *et al.*, 1987) or by electroelution of the P1 band from SDS-PAGE (Jacobs *et al.*, 1987*b*, 1988*b*). By both procedures the yield of P1 is about 1% of the total *M. pneumoniae* cell protein, making the product a most expensive one. This naturally led to the idea of expressing the P1 and MgPa genes in *E. coli*. Unfortunately, this approach encountered a serious problem. The usage by *Mycoplasma*, *Ureaplasma* and *Spiroplasma* species of the universal stop codon UGA as a tryptophan codon (for references see Blanchard, 1990) hinders the expression in *E. coli* of mycoplasmal messages. This is particularly true for genes like P1 and MgPa, which carry more UGA than UGG codons (Table 1), explaining the production of truncated rather than complete adhesin proteins in *E. coli* transfected with these genes (Trevino *et al.*, 1986; Schaper *et al.*, 1987). Possible ways to overcome this problem include the use of suppressor *E. coli* strains found to be effective in the expression of a spiroplasmal DNA methylase gene (Renbaum *et al.*, 1990). Another way is to convert *in vitro* TGA to UGG in mycoplasmal genes by site-directed mutagenesis, an approach employed by Li *et al.* (1990) in the development of a recombinant adenovirus vaccine containing P1 gene

inserts (see below). Yet, despite the expected difficulties resulting from the UGA codon problem, fusion proteins containing large moieties of *M. pneumoniae* (Trevino *et al.*, 1986; Ogle *et al.*, 1991) and *M. genitalium* (Razin & Yogev, 1988; Yogev & Razin, 1990) proteins can be obtained, using the expression vector λ gt 11. It should be mentioned that the mycoplasmal gene inserts may contain not only UGA but also the conventional UGG codons for tryptophan. In this way fusion proteins with antigenic moieties common to the adhesins of both *M. genitalium* and *M. pneumoniae* have been obtained (Yogev & Razin, 1990).

The main antigenic components of *M. pneumoniae* cells are proteins and glycolipids. Antibodies directed against the P1 protein are regularly found in patients with acute *M. pneumoniae* infections (Jacobs *et al.*, 1987*a*; Vu *et al.*, 1987). Since the P1 adhesin functions also as a major antigen of *M. pneumoniae* cells, anti-adhesin antibodies are expected to inhibit the adherence of *M. pneumoniae* to host cells and protect against infection, promoting the idea of using the P1 protein as a vaccinogen (Razin, 1986). Guinea pigs have been immunized with the purified P1 protein systemically by intraperitoneal injections (Jacobs *et al.*, 1988*c*) or intranasally in order to induce a more local antibody response (Jacobs *et al.*, 1988*a*). These animal studies have confirmed that the P1 protein is an effective antigen, eliciting strong systemic and local antibody responses, so that the vaccinated animals should be protected against a subsequent infection with live *M. pneumoniae*. In fact, the number of mycoplasmas in bronchial washings of immunized guinea pigs challenged with virulent *M. pneumoniae* was much smaller compared to unvaccinated challenged animals. However, lung tissues of the immunized guinea pigs showed no reduction of lympho-histiocyte infiltrations around bronchi and small vessels. In some cases, the immunized and challenged animals revealed an exacerbation of the cell-mediated response, indicating that enough mycoplasma antigen was still present to maintain the cellular immune response. Interestingly, exacerbation of clinical syndromes is also observed in children and young adults with a prior history of a *M. pneumoniae* disease and subsequent infection with *M. pneumoniae*. Hence the P1 adhesin, as well as the MgPa adhesin of *M. genitalium* (Jacobs *et al.*, 1991), is a potent immunogen inducing a strong antibody response but also a specific but undesired strong cellular response, making vaccination with purified P1 protein potentially harmful. Furthermore, the animal studies as well as observations showing that immunocompetent persons with significant titres of anti-P1 antibodies are not protected from a second *M. pneumoniae* infection also discourage the use of the P1 protein as a vaccine candidate.

The answer to the above, somewhat unexpected, results was found on testing of patients with *M. pneumoniae* disease and of animals experimentally infected with *M. pneumoniae* (Jacobs *et al.*, 1985; Jacobs *et al.*, 1988c). Less than 3% of the specific anti-*M. pneumoniae* antibodies in the sera of men or animals exhibiting high anti-P1 antibodies showed attachment-inhibiting activity. It was, therefore, suggested that the P1 protein induces anti-P1 antibodies which are mostly not directed to the major adherence-mediating epitopes but to other more immunogenic sites of the P1 amino acid sequence. To test this suggestion, patients' sera were examined for anti-*M. pneumoniae* antibody responses to overlapping synthetic octapeptides deduced from the amino acid sequence of the P1 protein (Jacobs *et al.*, 1990a). These mapping experiments clearly showed that most of the antibodies in the patients' sera did not react with the P1 regions involved in adherence. One of the two immunodominant regions with a significant number of anti-P1 epitopes is clearly separated from the adherence-mediating D1-domain (Fig. 3). Within the second immunodominant region (the D2-domain), an amino acid sequence was found which showed both binding of patients' antibodies and binding of adherence-inhibiting antibodies; other epitopes in this region showed no overlap of this type (Fig. 3). It thus appears that the adherence-mediating epitopes may be less antigenic in man than the other P1 epitopes. Therefore, repeated infections are necessary to build up a satisfactory level of antibody.

With this information available, the best strategy to follow appears to be the development of a P1 vaccine which should induce, on the one hand, a strong adherence inhibiting activity, but on the other hand should induce only a low but specific cell-mediated immunity so as to avoid adverse effects, i.e. the exacerbation of cell responses during a second contact with the *M. pneumoniae* antigen. Two approaches to vaccine development are now under study. One approach is based on the idea of establishing a mainly local immunity using a recombinant P1-adenovirus oral vaccine (Li *et al.*, 1990). The live recombinant viruses will replicate in the mucosal layer of the gut and continuously produce the P1 immunogen. Activated lymphocytes produced at the gut-associated lymph nodes would migrate to the respiratory tract, and protect the immunized subjects. The expression of specific P1 epitopes by the recombinant virus can be controlled by the P1 gene sequences incorporated into the recombinant. Obviously, selection of the sequences should include adherence-mediating epitopes, while avoiding too many T-cell-stimulating epitopes. The second approach is directed at developing a synthetic vaccine constructed of short amino acid sequences wrapped up in

liposomes for intranasal application (Jacobs, 1990). The peptides selected will be those characterized as adherence-mediating epitopes. In addition, the vaccine should include a minimum sequence of the P1 protein consisting of one T-cell-stimulating epitope, linked to a B-cell epitope which together will be able to induce an antibody response. Linking covalently the B- and T-cell-stimulating amino acid sequence to the less immunogenic adherence-mediating sequences is expected to enhance antibody response to the latter. On screening the P1 protein sequence for T- and B-cell epitopes, a sequence of 11 amino acids, which includes components of both a T- and a B-cell epitope was characterized (Jacobs *et al.*, 1990b), so that both the adherence-mediating epitopes and the T- and B-cell epitope are now available for vaccine construction and trial.

Adhesins as diagnostic probes

The P1 and MgPa adhesins have the potential to serve as diagnostic probes for the following reasons: (i) the P1 and the MgPa proteins are immunodominant antigens, eliciting strong humoral responses; (ii) both proteins are exposed on the mycoplasma cell surface, and can thus be easily labelled with anti-adhesin antibodies; and (iii) the nucleotide sequence of the two adhesin genes is known, facilitating the use of specific sequences of these genes as probes in polymerase-chain reactions (PCRs).

The conventional serodiagnostic approach based on complement fixation with the glycolipid antigen of *M. pneumoniae* suffers from the low specificity of this antigen (Tully & Baseman, 1991; Jacobs, 1991). A search was therefore carried out to identify a specific *M. pneumoniae* protein antigen to replace the glycolipid. ELISA tests with purified P1 showed that this protein may serve as an adequate antigen, as antibody responses to the highly immunogenic P1 were found in all patients with *M. pneumoniae* infections (Jacobs *et al.*, 1986). Moreover, the major advantage is the much higher specificity of the P1 antigen as compared to the glycolipid extract (Jacobs *et al.*, 1987a). Although the P1 protein of *M. pneumoniae* shares homologous amino acid sequences with the MgPa protein of *M. genitalium*, cross-reactions between these two antigen preparations are weak (Jacobs *et al.*, 1991). Now that the amino acid sequences of both adhesins are known, as well as their common and the different epitopes, it is possible to synthesize oligopeptides to be used as specific antigens in serological tests (Jacobs *et al.*, 1990a; Hirschberg *et al.*, 1991). Oligopeptides with epitopes common to *M. pneumoniae* and *M. genitalium* may be used to facilitate the serological screening of mycoplasma infections of the respiratory tract, considering that *M. genitalium* may also

be present there (Baseman *et al.*, 1988). Species-specific epitopes may be used for epidemiological purposes or for serodiagnosis of possible urogenital tract infections by *M. genitalium*.

For the more rapid diagnosis required for selecting effective treatment of the patient, two different diagnostic approaches are currently under clinical testing to replace the extremely slow and expensive culture techniques. The first approach is based on detection of *M. pneumoniae* in clinical specimens by specific anti-P1 monoclonal or polyclonal antibodies. These antibodies, coated onto ELISA plates, are used to capture P1 molecules out of solubilized material (nasopharyngeal aspirates, bronchial washings or sputum) from patients. The 'captured' P1 protein is detected with enzyme-conjugated anti-P1 or with anti-*M. pneumoniae* antibodies (Hirschberg & Holme, 1991; Gerstenecker & Jacobs, 1992). The second approach is using PCR to detect *M. pneumoniae* or *M. genitalium* in clinical specimens. Primers of P1 or MgPa gene sequences flanking the regions to be amplified are used for amplification of the target sequence (Bernet *et al.*, 1989; Jensen *et al.*, 1991). There can be little doubt that these new rapid diagnostic techniques will become a standard repertoire of the routine diagnostic laboratory. Thus basic research, focused primarily on mechanisms of adhesion and pathogenicity of mycoplasmas, is finding application now in the development of new strategies for diagnosis and prevention of mycoplasma diseases in man.

Conclusions

Concentrating research efforts on adhesion of *M. pneumoniae* and *M. genitalium* has paid off. The molecular properties of the major adhesins of these mycoplasmas, P1 and MgPa, as well as the chemical composition of host cell receptors have been defined. The topography of the large adhesin molecules embedded in the mycoplasma membrane, as predicted by computer analysis of amino acid sequences, has been supplemented by epitope mapping using mAbs acting on adhesin molecules *in situ*, or on synthetic oligopeptides corresponding to defined segments of the adhesin molecules. Regions of adhesin molecules inducing cytoadhesion-inhibiting antibodies have been defined in this way.

The definition of adhesin epitopes has opened the way for constructing *M. pneumoniae* vaccines based on the P1 molecule, or on defined portions of it. As the injection of entire P1 molecules causes exacerbation of host cell responses in experimental animals, efforts are now being put into selecting P1 peptide sequences having no

adverse effects. This may be achieved by synthesizing oligopeptides with the required P1 epitopes, or by inserting the appropriate P1 gene segments into a recombinant adenovirus, to serve as a live vaccine. Synthetic P1 oligopeptides and species-specific P1 and MgPa gene sequences are also in the process of being introduced as probes in the diagnosis of *M. pneumoniae* and *M. genitalium* infections.

Cytoadhesion of the mycoplasmas is a complex, multifactorial process involving, in addition to the major adhesins, a number of 'accessory' membrane proteins acting in concert with cytoskeletal elements to facilitate the lateral movement and concentration of the adhesin molecules at the attachment tip organelle of *M. pneumoniae* and *M. genitalium*. Information on this apparently complex network is meagre, and much remains to be resolved.

Oxidative damage to the host cell membrane by peroxide and superoxide radicals excreted by the adhering mycoplasmas appears to be experimentally well-substantiated. However, the possibility that the close contact of the mycoplasma membrane with that of the host leads to local and transient fusion of the two membranes, or to exchange of membrane components is intriguing, and should be investigated.

M. pneumoniae and *M. genitalium* are not the only mycoplasmas pathogenic to man, and there are many animal mycoplasmas that depend on adhesion to host tissues for colonization and infection. It can be expected that the lessons learned from the intensive studies on *M. pneumoniae* and *M. genitalium* adhesion will be applied to define the adhesion systems in other pathogenic mycoplasmas.

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