SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Details of experiments to determine the localization of glycolytic enzymes on the surface of *M. pneumoniae* cells.

For fluorescence microscopy, mycoplasma cells were cultured in cavities of a chamber slide (Thermo, Dreieich, Germany) and treated as described (1). Briefly, fixed mycoplasmas with formaldehyde (10% in PBS, Merck) for 20 min were incubated with a mixture of guinea pig antiserum against a glycolytic enzyme and rabbit antiserum to the TX 100-insoluble fraction (representing the membrane-associated protein fraction (2)) of *M. pneumoniae* total proteins (1:250 each). A mixture of FITC-labeled anti-guinea pig IgG (Sigma) and TRITC-labeled anti-rabbit IgG (Sigma, 1:500 each) antibodies were added for detection. Pre-immune serum from a guinea pig and antiserum to total proteins of *M. pneumoniae* were used as negative and positive controls, respectively. The fluorescence signals of the cells were checked at 400x using a fluorescence microscope (Axioskop, Carl Zeiss, Jena, Germany).

Furthermore, surface association of the proteins was tested with the colony blot technique as described (1). Briefly, nitrocellulose (Schleicher & Schuell, Dassel, Germany) with blotted mycoplasma colonies was treated with guinea pig antibodies to glycolytic proteins, to total proteins of *M. pneumoniae* (positive control), to Eno of *M. pneumoniae* (cytosolic protein (1, 3)) and to pre-immune serum (negative control; 1:250 each). Detection was performed with HRP-conjugated anti-guinea pig IgG (1:750).

After analyzing the cleavage sites of glycolytic enzymes for chymotrypsin (http://web.expasy.org/peptide_cutter/), mild proteolysis of *M. pneumoniae* cells was carried out. Freshly grown mycoplasmas were harvested as described and adjusted to a protein concentration of 200 µg/ml PBS. The cells were centrifuged (13000 g, 5 min) and treated with 0, 5, 10 and 40 µg of chymotrypsin (Sigma) in PBS for 30 min at 37 °C. After a further centrifugation step (13000 g, 10 min), the pellet was resuspended in 100 µl sample buffer (3)

and boiled for 10 min. The proteins were separated by SDS-PAGE and blotted to nitrocellulose. Membranes were incubated with guinea pig antisera to glycolytic enzymes (1:250 each). Sera to cytosolic Eno of *M. pneumoniae* (negative control) and the surface-exposed C-terminal part of P1 adhesin were used as negative and positive controls. Proteins and digestion products were detected using peroxidase-conjugated anti-guinea pig IgG (1:750).

LEGENDS TO FIGURES

FIG S1 Glycolysis in *M. pneumoniae* (after (4), modified) and glycolytic enzymes included in the study (dashed frames: proteins investigated in previous studies, see Table 1).

FIG S2 Concentration-dependent binding of human plasminogen (Plg) to immobilized surface-displayed glycolytic enzymes of *M. pneumoniae* and to BSA (15 µg/ml each) as measured by ELISA. Bound Plg was detected by rabbit anti-Plg and HRP-conjugated anti-rabbit IgG. Error bars indicate standard deviation of eight replicates of a representive experiment.

FIG S3 Influence of pre-incubation of human Plg with recombinant proteins on binding of Plg to recombinant proteins. Wells of ELISA plates were coated with recombinant proteins (15 µg/ml) derived from surface-displayed glycolytic enzymes of *M. pneumoniae*. Plg (2,5 µg/ml) was pre-incubated with each of these recombinant proteins (15 µg/ml; room temperature, 90 min). Mixture was added to immobilized recombinant proteins and incubated for 2 h at 37 °C. Bound Plg was detected with rabbit anti-Plg (1:2,500) and anti-rabbit IgG (1:1,000). Binding of Plg after incubation with BSA was used as negative control and set to 100 %. Data of wells incubated with mixture of Plg and recombinant protein are calculated as percentages of negative control. Mean and standard deviation of eight replicates.

FIG S4 Detection of glycolytic enzymes of *M. pneumoniae* in the supernatant of 4-days old bouillon cultures. Supernatant (50 ml) was filtered (0.22 µm) to remove non-adherent bacteria

and concentrated to 1,000 µl by centrifugation through Vivaspin columns (MWCO: 10,000). Concentrated supernatant was used to coat wells of ELISA plates and occurrence of glycolytic enzymes was confirmed by incubation with guinea pig antisera to recombinant proteins (1:100) followed by HRP-conjugated anti-guinea pig IgG (1:750). Antiserum to total proteins of *M. pneumoniae* (M.p.) was used as positive control. Mean and standard deviation of two independent experiments with eight replicates each are shown.

FIG S5 Results of treatment of freshly grown *M. pneumoniae* cells with chymotrypsin (30 min; 37 °C). Digestion products of glycolytic enzymes were detected by incubation of SDS-separated and blotted whole proteins of cells with guinea pig antisera to recombinant proteins and anti-guinea pig IgG (lane 1: 0 µg; lane 2: 40 µg chymotrypsin). Antisera to recombinant protein P12 (surface-localized C-terminal part of P1 adhesin), to Eno of *M. pneumoniae* and pre-immune serum (PIS) were used as positive and negative controls, respectively.

TABLES

TABLE S1 Oligonucleotides used in the study (underlined: vector-specific sequence; double underlined: mutation of TGA to TGG)

Target	Oligonucleotide	Sequence (5' → 3')	Description
AckA	MpAckAf1	GAA TAA TAC CTA CAG CTG TCG	Amplification,
	MpAckAf2	TAG CGC CAC TGC ACA ACA AG	cloning and
	MpAckAf3	ATT GGT TAT GTA GCA GAA CAG G	sequencing
	MpAckAr	GAC TTG GTA CTT ACT CCC ATC	
	MpAckAVf	GAC GAC GAC AAG ATG AAC GAC AAC AAA ATT TTA GTA G	
	MpAckAVr	GAG GAG AAG CCC GGT CTA TTT TTG AGT TAG TCG AAT GGA	
Pfk	MpPfkf1	GAT CTC GAC AAA TTC CGC C	Amplification,
	MpPfkf2	CTA AAT TGC TAA GCG AGT TGG G	cloning and
	MpPfkr	GTA AGC TGG GTC ATC TAA CAT TG	sequencing

	MpPfkVf	GAC GAC GAC AAG ATG AGT CCA AAA ACA ACC	
	MpPfkVr	GAG GAG AAG CCC GGT TTA AAT AAT ATT TTT ATT AAT GAC TG	
Fba	MpAldof1	CAG TAC CGG TGA TGA AAT TGT G	Amplification,
	MpAldof2	GCT CGC ATT TAC CCT TTC AAG	cloning and
	MpAldor	GGT TGA TTT ACC AAC GTT GGG	sequencing
	MpAldoVf	GAC GAC GAC AAG ATG CTA GTA AAC ATC AAA CAA ATG	
	MpAldoVr	GAG GAG AAG CCC GGT CTA AGC CTT ATT GGT TGA ACC	
	MpAldoM1	P-CCG ACA ACT GGA AGG GTT TAA ACT ATG	MMR*
Eno	MpEnof1	CAC CAC CAA AAT AGA TTT GTT TC	Amplification,
	MpEnof2	CAA TAA GGC TAA GTT AGG TGC	cloning and
	MpEnof3	CCA GGT AGT GAC ATT GCG	sequencing
	MpEnof4	CGA AGA CAC GAC AAT TGC TG	

	MpEnor	GAT AAT GAG GGC AAT TGC TTT AG	
	MpEnoVf	GAC GAC GAC AAG ATG AGT GCA CAA ACT GGA ACC	
	MpEnoVr	GAG GAG AAG CCC GGT TTA AGC TTT TTGCGG TTT AAT G	
	MpEnoM1	P-CAA TAA TGC TAA GTA CTT AGG TTG GAAC	MMR
Pgi	MpPgif1	CCT TGT GAC TTT ATG AGA GCT ATC	Amplification,
	MpPgif2	GCA GTT GCT AGA GCA ATT AAG G	cloning and
	MpPgif3	CAC GAC AAA GAA GCT CAA AAT AG	sequencing
	MpPgir	CGT CAT AGT GGA TTA AGC GC	
	MpPgiVf	GAC GAC GAC AAG ATG GAA AGT AAA TGG TTA ACA G	
	MpPgiVr	GAG GAG AAG CCC GGT CTA TTC ACG ACC AAG TTT AGC	
	MpPgiM1	P-GGT TTT TCA TGG GCT TTG GTA GTA AC	MMR
	MpPgiM2	P-TGC TTT GCG TCA CTG GCT TTA C	

GapA	MpGapAf	GAA TTA CTC GCC ATA GGT TAA AG	Amplification,
	MpGapAr	CTG AAA GTC AAA CGC TTG G	cloning and
	MpGapAVf	GAC GAC GAC AAG ATG CTA GCA AAG AGT AAG ACT ATC	sequencing
	MpGapAVr	GAG GAG AAG CCC GGT TTA AAG CTT GGC ACA ATA GTT AAC	
	MpGapAM1	P-CTA ACC TGC CAT GGG CAG AAC AC	MMR
Ldh	MpLdhVf	GAC GAC GAC AAG ATG AAG AGT CTT AAA GTA GCA CTC ATT G	Amplification,
	MpLdhVr	GAG GAG AAG CCC GGT TTA TAG TTT AGC TAA CTT AAT GTT GTC CTT	cloning and
			sequencing
Pta	MpPtaf1	CTG GTG GTG GTG CAT CTT TAG C	Amplification,
	MpPtar	GCA CCC GAC AAC CTC AAA C	cloning and
	MpPtaVf	GAC GAC GAC AAG ATG AGT GTT ATT GAT CTT TTA AAA C	sequencing

	MpPtaVr	GAG GAG AAG CCC GGT TTA TTT AAG TGT TTG CGC TG	
	MpPtaM1	P-CCT GAA GGT TGG TCG CCA	MMR
FruK	MpFruKf1	GTC ATA ACC TGC TTG TAG TGC G	Amplification,
	MpFruKf2	GAT TGC TTA CCT TAA GGC TAA CG	cloning and
	MpFruKr	CTT TGG CTG TTT GGC TAG TTC	sequencing
	MpFruKVf	GAC GAC GAC AAG ATG CTT AAC CAC AAC AGT AAA GTT TGG ATA G	
	MpFruKVr	GAG GAG AAG CCC GGT TTA TAA ATA GTA ATA ATT AGT AGC TTT TAG CAC	
Pgk	MpPgkf1	CGA ATC CTC TTA CGT AAA CCA G	Amplification,
	MpPgkf2	GAT GTG AAT GAG GCT GGT G	cloning and
	MpPgkf3	GGA GGA AGA CTT AGT CCA AAC C	sequencing
	MpPgkr	CCA GCA CTT TGA ACA CGT TG	
	MpPgkM1	P-CAG TTC TGG GCA TCA TTG GGT G	MMR

	MpPgkM2	P-CAA TGG CCA GCA GAA CTT CAG	
Pgm	MpPgmf1	GCA AGG CAT CTT TGG ATG TC	Amplification,
	MpPgmf2	CAG TCA CAT GGA CCA TTT GTA TG	cloning and
	MpPgmf3	GTC ACA CAT GCT CTT TCA AAC AG	sequencing
	MpPgmf4	CTG ATA TGG TAG GTC ATA CTG GTA AC	
	MpPgmr	GCC ATC AGA TAC ACC GAT ACC	
	MpPgmVf	GAC GAC GAC AAG ATG CAT AAG AAG GTC TTA CTA GCC	
	MpPgmVr	GAG GAG AAG CCC GGT TTA CTT TTT ACT TAT TAA AAG GGA G	
ManB	MpManBf1	GAT AAG ACC CAG TTA TTC TGC	Amplification,
	MpManBf2	GTG TGA TGT GAT TAC CTC TAT G	cloning and
	MpManBf3	TTA CAT GGT ACT TCG GTG AAG	sequencing
	MpManBf4	GAC AAT GAA CTA GTA GTG G	

	MpManBf5	CCT AGT CGA ATA TGT CTT GG	
	MpManBVf	GAC GAC GAC AAG ATG AAC AGT AAC GCA TAC TTG	
	MpManBVr	GAG GAG AAG CCC GGT CTA AGC TTT ATC GAG GTT GAG C	
	MpManBM1	P-TAA GGA TAC CTG GGA GTT AGC G	MMR
	MpManBM2	P-GCG ATT GCC GAA TGG AAT CCG	
	MpManBM3	P-TGG TGA TTA CTA TAA CTG GAC GG	
PdhA	MpPdhAf1	GTT AGC AGC CGT GTT GCA AG	Amplification,
	MpPdhAf2	CAA CGA ACA GTT AAA GCA CGC	cloning and
	MpPdhAf3	CAA AAC GCT GCC CAT TAA C	sequencing
	MpPdhAf4	GCG AGT TAC GAA GCA ATG C	
	MpPdhAr	GAT AGA TCC ATT GCG TTA CCC	
	MpPdhAVf	GAC GAC GAC AAG ATG GCA ATT TTG ATT AAA AAC	

	MpPdhAVr	GAG GAG AAG CCC GGT TTA GTC TTT AAA GTA TTT TTT AGC	
	MpPdhAM1	P-GCT TGT TTG GCA ACG TGC TG	MMR
	MpPdhAM2	P-GCT TTT ACT TTA CTG GAA CGG TAA C	
	MpPdhAM3	P-CCA TTC ACA AGT GGA ACT CGG	
	MpPdhAM4	P-CTT CTC TTG GCG TCA AGG TC	
PdhB	MpPdhBf1	GTT GAC TCC TGA TCT AGC GC	Amplification,
	MpPdhBf2	CAT GCC AAT GGG TGG TG	cloning and
	MpPdhBf3	GGG TAA CGC AAT GGA TCT AG	sequencing
	MpPdhBf4	GGA CAA GGG CAT TGA ACT C	
	MpPdhBr	CTA GGT GCA ATT ATT CTA ACA AAC	
	MpPdhBVf	GAC GAC GAC AAG ATG TCA AAA ACA ATT CAA GCA AAT AAC	
	MpPdhBVr	GAG GAG AAG CCC GGT TTA CTT TAA AAG TTG GTT AAC AGC TTC	

	MpPdhBM1	P-CGT ACC ATT TCC CCT TGG GAC AAG	MMR
PdhC	MpPdhCf	CAG CTA ACT GCA GTA AAT GCA AC	Amplification,
	MpPdhCr	CAC ACG CCA CCA AAG TAT TC	cloning and
	MpPdhCVf	GAC GAC GAC AAG ATG GCA AAT GAG TTT AAG TTC AC	sequencing
	MpPdhCVr	GAG GAG AAG CCC GGT CTA AGC TAC TTC TAA ATC AAT TAA TTC	
PdhD	MpPdhDf1	CAT GGA AGA ACG TGT TGT GC	Amplification,
	MpPdhDf2	CAA GAT TGT CGA TTA CCT CC	cloning and
	MpPdhDr	CTT CTA ACG CTG CGT TGA AG	sequencing
	MpPdhDVf	GAC GAC GAC AAG ATG AAT TAC GAT CTC ATT ATT ATA G	
	MpPdhDVr	GAG GAG AAG CCC GGT CTA TTT AAA GTG ATC AAA AAG AGC	
	MpPdhDM1	P-GTA GCA CTT ACC TGG AAC CAA TTG	MMR

Pyk	MpPykf1	GAT CGC AGC GCT TTA ATT G	Amplification,
	MpPykf2	CAC TTA CAA CAT GGT AAA CGA TG	cloning and
	MpPykf3	GAG ATC CCA TAT TAC AAG GTG C	sequencing
	MpPykr	GAC GAT ATG GAG GTT GCA GC	
	MpPykVf	GAC GAC GAC AAG ATG ATT CAC CAC CTAAAA CG	
	MpPykVr	GAG GAG AAG CCC GGT TTA TAA GCT AAT GAT CTT ATT GTG AA	
	MpPykM1	P-CTA TGG ACG CTA GCA ATG TTA GAT GAC CCA	MMR
	MpPykM2	P-CAA GGT GCC TTA CTG GCA ACG	
	MpPykM3	P-CAG TGA GTT TTG GAA GCA GGT GG	
Tkt	MpTktf1	CCT AAT TGC CCA ACT AGC C	Amplification,
	MpTktf2	GTG ATG GTG ATC TCC AGG AAG	cloning and
	MPTktf3	CAC AAG CAG GTC ATA GCA CG	sequencing

	MPTktf4	CAA GTC GGT GGT GAT GGA C	
	MPTktR	GTA GGA ATA TAG TCC TTA AAC GAA C	
	MpTktVf	GAC GAC GAC AAG ATG AAA AAC CTG TTT GCT TG	
	MpTktVr	GAG GAG AAG CCC GGT TTA ACT CTT TAG AGT TTT AAT CAA C	
	MpTktM1	P-CAA CAT GGG GTG GAA TTA CC	MMR
	MpTktM2	P-GTT TAT TAA TTG GAC GGA AAA CGA TTA	
	MpTktM3	P-CAG GTT TGG CGG CCT TGT	
TpiA	MpTpiAf1	GCA CCT GAT AAA GTA GTC CCA C	Amplification,
	MpTpiAr	GAG TTA CCG ATT TGT CCT TGT G	cloning and
	MpTpiAVfR	GAA GGA GA TAT ACA TAT GCG TAC GAA ATA CCT AAT TG	sequencing
	MpTpiAVrR	GTG ATG GTG GTG ATG ATG TGC ATA TAC TTG TGC CAT TAC	
	MpTpiAM1	P-GGT AAC TGG AAG ACA AAT AAG GAT TTA CAC	MMR

	MpTpiAM2	P-GCT TAC GAA CCA ATT TGG GCA AT	
pET vector	ExpNF	GTA TGA AAG AAA CCG CTG C	Sequencing

* - multiple mutation reaction (5)

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