MHC Class II Immunoproteomic Analysis of Infected Murine Dendritic Cells Reveals Unique and Overlapping Epitopes between *Chlamydia trachomatis* and *C. muridarum*[1]

Karuna P. Karunakaran[*], Xiaozhou Jiang[*], Kyung-Mee Moon[†], Caixia Shen[*], Hong Yu[*], Leonard J. Foster[†], and Robert C. Brunham[*, 2]

[*]British Columbia Centre for Disease Control; [†]Department of Biochemistry and Molecular Biology, Centre for High-Throughput Biology, University of British Columbia Vancouver, B.C., Canada.

[1]This work was supported by National Institutes of Health Grant R01AI076483 (to RCB and LJF). LJF is the Canada Research Chair in Quantitative Proteomics.

[2]**Corresponding author:** BCCDC, 655 West 12th Avenue, Vancouver, BC V5Z 4R4, Canada. Tel: 604-707-2409, Fax: 604-707-2401, E-mail: robert.brunham@bccdc.ca

Running title: Immunoproteomic analysis of *C. trachomatis* and *C. muridarum*

Key words: *Chlamydia*, Immunoproteomics, MHC, T cell, Epitope, Peptide, Antigen, Vaccine

ABSTRACT

Protective immunity to *Chlamydia* is T cell-mediated, requiring MHC class II presented antigens. In this study an immunoproteomic approach was used in which *Chlamydia*-derived peptides presented by immunoaffinity purified MHC class II molecules on the surface of infected murine dendritic cells (DCs) were identified by tandem mass spectrometry. DCs from Balb/c mice infected with *C. muridarum* yielded 14 MHC class II binding peptides from 11 different *C. muridarum* proteins. Only one of the 11 proteins was shared in common with the previously identified 16 *C. muridarum* proteins that generated MHC class II binding peptides using DCs from C57BL/6 mice. On the other hand C57BL/6 DCs infected with *C. trachomatis* serovar D yielded 60 MHC class II binding peptides derived from 27 proteins, 10 of which were shared between the *C. muridarum* and *C. trachomatis* proteome. Three shared T cell antigens belonged to the polymorphic membrane family of proteins, which generated MHC class II binding epitopes at distinct sequences within the proteins. We conclude that T cell immunodominance is determined by both MHC class II allelic selection and inherent properties of pathogen proteins. Immunodominant T cell antigens are potential vaccine candidates against human *C. trachomatis* infections.

INTRODUCTION

In women, sexually transmitted *Chlamydia trachomatis* infections are an important cause of pelvic inflammatory disease and reproductive sequelae including tubal infertility, ectopic pregnancy and chronic pelvic pain. *C. trachomatis* can also spread among children via fomites and flies to produce trachoma, a leading cause of infectious blindness in selected areas of the developing world. Public health measures to prevent and control sexually transmitted *C. trachomatis* appear to be failing as case rates continue to rise and efforts to control ocular *C. trachomatis* infection are not fully effective using current approaches such as the World Health Organizations's SAFE (surgery, antibiotics, facial cleanliness and environmental improvements) strategy (1). Thus, developing a *Chlamydia* vaccine would be a valued solution to control *C. trachomatis* infections.

The results from vaccine trials against ocular *C. trachomatis* infections in the 1960s with inactivated, whole-organism vaccines suggested that the development of protective immunity is feasible in humans (2). Although the trials demonstrated short-term partial protection, further development was halted because non human primates experienced enhanced inflammation upon re-infection due to vaccine primed immunopathologic responses. As such, the focus among researchers shifted toward the development of subunit vaccines that may bypass the damaging immunopathologic response incited by the whole cell vaccine.

Through immunobiological studies of *C. muridarum* infection in mice and immunepidemiological studies of *C. trachomatis* infection in human, *Chlamydia* immunity is now understood to be CD4 T cell-mediated, requiring MHC class II

presented antigens and dependent on IFN-γ and TNF-α secretion. T cell immunity may be enhanced with local antibodies (3).

Dendritic cells (DCs) are at the centre of initiation of T cell mediated immune responses (4). They capture antigen in the periphery and migrate to regional lymph nodes where they present processed antigen on MHC molecules to naïve T cells to induce, amplify and polarize T cell mediated immune responses. Since T cells mainly recognize protein antigens, protective vaccine candidates are to be found within the proteome of an organism. Through genomic analysis, the proteomes for different species of *Chlamydia* have been fully inferred (5, 6). An approach called immunoproteomics (7), in which peptides presented by immunoaffinity purified MHC molecules are identified by tandem mass spectrometry (MS/MS) allows genomic information to guide the delineation of the immunoproteome of an organism.

We used an immunoproteomics here to identify *Chlamydia* epitopes presented by MHC class II molecules from bone marrow derived DCs (BMDCs) infected with *C. muridarum* under different experimental conditions (8, 9). *Chlamydia*-specific CD4 T cells harvested from mice recovered from *C. muridarum* infection recognized these MHC class II-bound peptides *in vitro* (10) and the source proteins of these MHC class II-bound peptides accelerated clearance of *C. muridarum* genital tract infection when formulated with a Th1 polarizing adjuvant consisting of cationic liposome and modified mycobacterial cord factor (11).

We previously performed three immunoproteomic experiments in C57BL/6 mice to identify the MHC classII-bound peptide repertoire of *C. muridarum* (8, 9). In total 64 peptides of 19 unique epitopes derived from 17 *C. muridarum* source proteins were

identified using the three different experimental conditions. Eight T cell antigens (ClpP, TC0420, PmpE/F-2, PmpG, FabG, Aasf, Gap and RplF; Table I) were initially identified as presented by MHC class II molecules when BMDCs were infected with *C. muridarum* for 24 hours (8). Eight T cell antigens (Tsf, Ide, PmpE/F-1, PdhC, Tuf, DadA, FusA, and DsbD; Table I) were subsequently identified when BMDCs were infected with *Chlamydia* for 12 hours (9) including 5 of the original 8 antigens (ClpP, TC0420, PmpE/F-2, PmpG, FabG; Table I). The third experimental condition used dead instead of viable EBs and generated only three antigens, one of which was a new T cell antigen (AtpE; Table I) showing that replicating organism promoted better antigen processing and presentation than nonviable organism (9).

C. muridarum is a mouse-adapted strain of *Chlamydia* whose genome is highly syntenic to that of the human strain *C. trachomatis* with most genes being orthologs among the two species. The usefulness of the T cell antigens identified in the mouse strain *C. muridarum* for developing a human *Chlamydia* vaccine is dependent on the assumption that T cell antigens are common between the murine and human strains. In this study we compared the *C. muridarum* immunoproteome in two different inbred strains of mice to examine the impact of MHC class II alleles on selecting T cell antigens and then investigated the *C. trachomatis* immunoproteome using infected murine DCs by comparing the findings to the *C. muridarum* immunoproteome.

METHODS

Chlamydia

C. muridarum strain Nigg and *C. trachomatis* serovar D were grown in HeLa 229 cells in Eagle's essential medium [(MEM), Invitrogen] supplemented with 10% FCS. Elementary bodies (EBs) were purified from HeLa cells on discontinuous density gradients of Renografin-76 (Squib Canada) as described previously(12).

Mice

Female C57BL/6 and Balb/c mice (8 to 10 weeks old) were purchased from Charles River Canada (Saint Constant, Canada). The mice were maintained and used in strict accordance with University of British Columbia guidelines for animal care.

Generation of BMDCs

Bone marrow derived dendritic cells (BMDCs) were generated as previously described (13). Briefly, bone marrow cells flushed from the femurs of 8-10 week-old female C57BL/6 or Balb/c mice were cultured at 7 x 10^5 cells/ml in 15 cm Petri dishes (Falcon) containing IMDM supplemented with 10% FCS, 15 ng/ml GM-CSF and 5% IL-4 culture supernatant of Hybridoma X63 (provided by Dr F. Melchers, Basilea Institute, Basilea, Switzerland). On day 7, nonadherent cells were harvested. Nonadherent cells were 70-80% CD11C positive and expressed moderate levels of costimulatory molecules CD80, CD86 and CD40 as well as MHC class I and class II molecules as determined by FACS analysis.

Purification of MHC class II-bound Peptides

MHC class II-bound peptides were purified as described previously (8). Briefly, 5 x 10^9 immature BMDCs were infected at a 1:1 multiplicity of infection with *C. muridarum* or *C. trachomatis* serovar D for 12 or 24 hours. BMDCs were then solubilized in lysis buffer (1% CHAPS, 150mM NaCl, 20 mM Tris-HCl, pH 8, 0.04% sodium azide, protease inhibitors). MHC class II molecules were isolated using allele-specific anti-MHC monoclonal antibody affinity columns containing the monoclonal antibodies Y-3P (specific to I-A MHC class II allele of both C57BL/6 and Balb/c) and 14-4-4S (specific to I-E MHC class II allele of Balb/c). Purified MHC class II molecules were separated from the peptides using 0.2N acetic acid and subjected to ultrafiltration through a 10-kDa cut-off membrane to remove high molecular weight material (14).

Identification of MHC class II-bound Peptides

The MHC class II-bound peptides were further purified, concentrated, filtered and desalted using STop And Go Extraction tips (15). Hydrogenated or deuterated forms of formaldehyde were used with cyanoborohydride as a catalyst to reductively dimethylate primary amines of peptides essentially as described (16). Peptides were then analyzed by LC/MS/MS using an LTQ-Orbitrap Velos (Thermo Electron) on-line coupled to Agilent 1200 Series nanoflow HPLCs using nanospray ionization sources (Proxeon Biosystems, Odense, Denmark). Analytical columns were packed into 15 cm long, 75 µm inner diameter fused silica emitters (8 mm diameter opening, pulled on a P-2000 laser puller from Sutter Instruments) using 3 mm diameter ReproSil Pur C_{18} beads. LC buffer A consisted of 0.5% acetic acid and buffer B consisted of 0.5% acetic acid and 80%

acetonitrile. Gradients were run from 6% B to 30% B over 60 minutes, then 30% B to 80% B in the next 10 minutes, held at 80% B for five minutes and then dropped to 6% B for another 15 minutes to recondition the column. The Velos was set to acquire a full range scan at 25,000 resolution in the Orbitrap, from which the three most intense multiply-charged ions per cycle were isolated for fragmentation in the LTQ. Fragment spectra were extracted using DTASuperCharge and searched using the Mascot and, PEAKS algorithms against a database comprised of the protein sequences from mouse and *Chlamydia*.

RESULTS:

Identification of *C. muridarum* Derived MHC class II-bound Peptides from Balb/c mice

We used the immunoproteomic approach to identify *C. muridarum* derived MHC class II-bound peptides presented on BMDCs from Balb/c mice. Bone marrow cells were isolated from the femurs of mice, expanded *in vitro* and then infected with *C. muridarum* for 12 h. Infected BMDCs were lysed and MHC class II molecules isolated using allele-specific anti-MHC class II monoclonal antibody affinity columns. Peptides bound to MHC class II molecules are eluted with acid and separated using ultrafiltration. The purified MHC-bound peptides are analyzed using mass spectrometer and fragment spectra obtained are searched against *C. muridarum* database. A total of 14 peptides derived from 11 *C. muridarum* source proteins (RecD_2, PmpH, TC0285, NusA, Tarp, Swip, RecO, TC0825, DacC, and YggV; Table I) were identified. Only one protein, translational elongation factor, Tuf (Table I) overlapped with the protein antigens identified using BMDC derived from C57BL/6 mice. The two peptide epitopes in Tuf were different in sequence between C57BL/6 and Balb/c mice. These data demonstrated a clear correlation between MHC allele background and peptides presented.

Identification of *C. trachomatis* Derived MHC class II-bound Peptides from C57BL/6 mice

The gene content of the *C. trachomatis* genome is highly syntenic to that of *C. muridarum* with nearly all genes being orthologous. Therefore we characterized the MHC

class II-bound peptide repertoire presented by *C. trachomatis* serovar D. BMDCs derived from C57BL/6 mice were infected with *C. trachomatis* serovar D for 12 h. Sixty peptides derived from 27 different *C. trachomatis* proteins were identified (Table II). Similar to the peptide repertoire identified in *C. muridarum*, the chromosomal location of genes encoding source proteins of the *C. trachomatis* derived epitopes were spread uniformally through out the *C. trachomatis* genome. Interestingly, none of the source proteins for T cell antigens were encoded by genes located in the plasticity zone, the highly variable chromosomal region between *C. muridarum* and *C. trachomatis* genomes that encode several of the species and biovar specific virulence factors.

Ten Source Proteins Derived MHC Class II–bound Peptides Overlap between *C. muridarum* and *C. trachomatis* Proteome

Comparison of source proteins among the MHC class II-bound *Chlamydia* peptides from both C57BL/6 and Balb/c revealed 10 overlapping proteins from the *C. muridarum* and *C. trachomatis* immunoproteome and 9 when C57BL/6 alone was considered (Table III). Fifteen of the 27 source proteins identified in *C. muridarum* immunoproteome were conserved at greater than 85% amino acid identity with the *C. trachomatis* proteins. Six of the ten overlapping source proteins (FabG, PdhC, RsbV, DadA, RplF and GapA) fell within the highly conserved group with more than 85% amino acid identity while the amino acid identity of the remaining four source proteins (CT143, PmpE, PmpG, and PmpH) was less than 76% (Table III).

For T cell proteins which overlapped between *C. trachomatis* and *C. muridarum* we expected that similar or identical epitopes would be derived from shared proteins

which were highly conserved in sequence while different epitopes may be presented from proteins less conserved in sequense. Five of the six highly conserved proteins presented the same or very similar epitopes (identical: FabG, RsbV, DadA; similar: PdhC, RplF) although one source protein (GapA) from the highly sequence conserved group presented a completely different epitope despite its 95% amino acid sequence identity. Three of the four less conserved proteins (PmpE, PmpG and PmpH) presented distinct epitopes (Table III) located at different sequence positions within the surface exposed passenger domains of these proteins (Figure 1).

DISCUSSION

CD4 T cell-mediated adaptive immunity is essential for host defense against *Chlamydia* infection. The identification of epitopes presented by MHC class II molecules capable of inducing protective immunity is a crucial prerequisite for the development of a T cell vaccine as recently demonstrated in a primate trial of a live attenuated *C. trachomatis* vaccine to prevent trachoma (17). The DC based immunoproteomic approach that we use in our laboratory directly identifies T cell epitopes presented by MHC molecules many of which engender protective immunity when tested as molecular immunogens. For instance when we tested 13 of the 27 T cell antigens identified in *C. muridarum*, 11 generated protective immune responses against *Chlamydia* genital infection in mice (submitted for publication in JI). These results argue that the immunoproteomic approach reveals antigens handled through *in vivo* physiological processing and presentation pathways which are relevant to protective immunity induced in animal model systems. Since T cell mediated protective immune responses are correlated with accelerated clearance of *Chlamydia* infection rather than with prevention of infection (18), T cell immunogens used in a *Chlamydia* vaccine must match antigens presented *in vivo* on MHC molecules during infection. In addition, the length of MHC class II-bound peptides reported in this study range between 10 and 38 amino acids and flanking regions extending beyond the binding grove are known to be relevant in antigen presentation to T cells (19). Importantly bioinformatic prediction of MHC class II-bound peptides focuses on identifying MHC anchoring motifs and is likely to miss the full sequence of such immunogenic peptides arguing for the role of immunoproteomics in discovering T cell antigens.

There are 924 and 894 proteins encoded by the genomes of *C. muridarum* and *C. trachomatis*, respectively, and in theory all of these could be processed and presented by antigen presenting cells. However, our findings show that less than 3% of the pathogen proteome is actually presented via MHC class II molecules following *Chlamydia* infection of the DCs. The reason for the restricted presentation of antigens by antigen presenting cells may relate to the sequestration of antigens within the *Chlamydia* inclusion and likely gives rise to immunodominance. Immunodominance of T cell antigens is of major importance to vaccinology and has been classified as intrinsic (i.e. differences in affinity for immune receptors) or extrinsic (i.e. competition that suppresses the response to one target in favor of another) (20).

The primary sequence of a protein is a major determinant of immunodominace since it determines antigen processing (protease susceptibility of the residues flanking the epitope), anchoring of the peptide into MHC binding pockets and affinity with the T cell receptor. Our data demonstrate that MHC allelic selection is clearly important to immunodominance as seen when the *C. muridarum* immunoproteome is compared between C57BL/6 and Balb/c mice. Sixteen proteins generated 64 peptide epitopes in C57BL/6 and 11 proteins generated 14 peptides in Balb/c. However, only one *C. muridarum* protein (Tuf) generated MHC class II binding epitopes in both strains of mice (Table I). The major outer membrane protein (MOMP) is an example to consider in detail since it is a well-known *Chlamydia* antigen which has been widely tested as a vaccine candidate (21). MOMP induces T cell mediated immunity in mice and multiple T cell epitopes have been previously identified within this surface exposed protein in both humans and mice (22, 23). Despite the use of multiple experimental approaches, none of

the *C. muridarum* immunoproteomic analyses we used identified MOMP derived peptides (Table I). However, a MOMP derived peptide was readily identified during the analysis of *C. trachomatis* immunoproteome (Table II). We compared the sequence of this peptide within *C. trachomatis* MOMP with that of *C. muridarum* and found that the peptide lies within the species specific epitope derived from the variable domain IV region which differs in sequence between *C. trachomatis* and *C. muridarum* MOMP (Figure 2). Presumably this MOMP epitope from *C. trachomatis* has anchoring residues enabling presentation on I-A^b molecules that *C. muridarum* lacks.

GroEL1 (Hsp60) is also a well studied *C. trachomatis* antigen (24). GroEL1 is the only documented antigen marker of protective *C. trachomatis* cellular immunity in humans (25). Similar to MOMP, GroEL1 was readily identified in the *C. trachomatis* immunoproteome but not in the *C. muridarum* immunoproteome (Tables I and II). The protein is highly conserved (whole protein 98% identical in amino acid sequence and the epitope region is identical) between *C. muridarum* and *C. trachomatis* suggesting that protein sequence is not the only factor for GroEL1 presentation via MHC. Rather the biology of *Chlamydia* replication in murine dendritic cells may differ such that GroEL1 enters the antigen processing and presentation pathways under the conditions of *C. trachomatis* replication in murine dendritic cells while *C. muridarum* GroEL1 may not.

Since 10 of 27 antigenic proteins in the *C. muridarum* and *C. trachomatis* were orthologs (Table III), immunodominance also appears to be determined by other inherent properties of pathogen proteins. Six of the 10 overlapping proteins (TC0261, TC0263, TC0264, TC0420, TC0707, and TC0801) have been demonstrated to confer protection in mice against *C. muridarum* geneital tract infection. The other four proteins were not

tested because they either had high homology to human homologs or were not unique to *Chlamydia* (submitted for publication in JI). Thus shared proteins in the two different bacterial species may have preference for entering the antigen processing and presentation pathways. Of particular interest, three of the overlapping T cell antigens belong to the polymorphic membrane family of proteins [Pmps (TC0261, TC0263, and TC0264)] which generated MHC class II binding epitopes at multiple sites within the sequence (Figure 1) suggesting that these proteins are uniquely capable of presenting to the host immune system via multiple MHC binding epitopes. Pmps belong to a group of proteins known as type V autotransporters comprising an N-terminal signal sequence, a passenger domain and a translocation unit (Figure 1) (26). *C. trachomatis* and *C. muridarum* genomes encode nine different Pmps (PmpA to PmpI) (5, 6). Identification of four Pmps as T cell antigens via the immunoproteomic analyses with three overlapping between the mouse and human *Chlamydia* proteome suggests that these proteins may have advantages over other groups of proteins in presenting to the adaptive immune system. This is supported by a recent quantitative proteomic analysis of *C. trachomatis* elementary bodies which revealed that the three Pmps we identified (CT869, CT871, and CT872) constituted 61% of the total Pmps (27). Thus high abundance of these proteins may play a role in favoring presentation. Additionally Pmps may also be favoured as T cell antigens because they may be part of outer membrane vesicles released by *Chlamydia* reticulate bodies that are engulfed by inclusion membrane vesicles which have the opportunity to traffic to the MHC class II antigen processing compartment in the cell (28, 29). In conclusion, the shared T cell antigens we have identified, in particular the

Pmps are likely relevant to *Chlamydia* immunobiology both in murine and human models and constitute potential vaccine candidates.

Table I: MHC class II-bound *C. muridarum*-derived peptides and their source proteins identified when murine bone marrow derived dendritic cells were infected with *C. muridarum* under different experimental conditions. Peptides identified in this study using Balb/c derived BMDCs are shown in bold.

Peptides	*Chlamydia muridarum* Locus#	Source Proteins	Protein Abbrev.	Mice strain used / References
AVPRTSLIF	**TC0021**	**Exodeoxyribonuclease V, alpha subunit**	**RecD_2**	**Balb/c, This study**
ISRALYTPVNSNQSVG	TC0050	Translation elongation factor Ts	Tsf	C57BL/6, (9)
IGQEITEPLANTVIA	TC0079	ATP dependent Clp protease, proteolytic subunit	ClpP	C57BL/6, (8)
LPLMIVSSPKASESGAA	TC0190	Metalloprotease, insulinase family	Ide	C57BL/6, (9)
SRALYAQPMLAISEA	TC0261	Polymorphic membrane protein E	PmpE	C57BL/6, (9)
AFHLFASPAANYIHTG	TC0262	Polymorphic membrane protein F	PmpF	C57BL/6, (8, 9)
NAKTVFLSNVASPIYVDPA ASPIYVDPAAAGGQPPA	TC0263	Polymorphic membrane protein G	PmpG	C57BL/6, (8, 9)
SPQVLTPNVIIPFKGDD	**TC0264**	**Polymorphic membrane protein H**	**PmpH**	**Balb/c, This study**
APILARLS	**TC0285**	**Hypothetical protein**	**TC0285**	**Balb/c, This study**
GGAEVILSRSHPEFVKQ	**TC0372**	**N utilization substance protein A**	**NusA**	**Balb/c, This study**
DLNVTGPKIQTDVD	TC0420	Hypothetical protein	TC0420	C57BL/6, (8, 9)
SPGQTNYAAAKAGIIGFS	TC0508	3-oxoacyl-(acyl carrier protein) reductase	FabG	C57BL/6, (8, 9)
EGTKIPIGTPIAVFSTEQN	TC0518	Pyruvate dehydrogenase	PdhC	C57BL/6, (9)
KPAEEEAGSIVHNAREQ	TC0584	V-type, ATP synthase subunit E	AtpE	C57BL/6, (9)
FEVQLISPVALEEGMR **GDAAYIEKVRELMQ**	**TC0596**	**Translation elongation factor Tu**	**Tuf**	C57BL/6, (9) **Balb/c, This study**
YDHIIVTPGANADIL	TC0654	Oxidoreductase, DadA family	DadA	C57BL/6, (9)
KLDGVSSPAVQESISE	TC0707	Ani-anti-sigma factor	Aasf	C57BL/6, (8)
GANAIPVHCPIGAESQ VFWLGSKINIIDTPG	TC0721	Translation elongation factor G	FusA	C57BL/6, (9)
KLEGIINNNNTPS	**TC0741**	**Translocated actin-recruiting phosphoprotein**	**Tarp**	**Balb/c, This study**
DPVDMFQMTKIVSKH	**TC0745**	**SWIB (YM74) complex protein**	**Swip**	**Balb/c, This study**
KIFSPAGLLSAFAKNGA	**TC0755**	**DNA repair protein**	**RecO**	**Balb/c, This study**
MTTVHAATATQSVVD	TC0792	Glyceraldehyde 3-phosphate dehydrogenase	Gap	C57BL/6, (8)
VKGNEVFVSPAAHIIDRPG	TC0801	Ribosomal protein L6	RplF	C57BL/6, (8, 9)
DDPEVIRAYIVPPKEP	**TC0825**	**Hypothetical protein**	**TC0285**	**Balb/c, This study**
LAAAVMHADSGAILKEK	**TC0839**	**D-analyl-D-alanine carboxypeptidase**	**DacC**	**Balb/c, This study**
SVPSYVYYPSGNRAPVV	TC0884	Thiol disulfide interchange protein	DsbD	C57BL/6, (9)
SMLIIPALGG	**TC0895**	**Nucleoside triphosphatase**	**YggV**	**Balb/c, This study**

Table II: MHC class II-bound *C. trachomatis*-derived peptides and their source proteins identified when murine (C57BL/6) bone marrow derived dendritic cells were infected with live *C. trachomatis* for 12 hrs.

Peptide	*Chlamydia trachomatis* Locus#	Source Proteins	Protein Abbrev.
YKLVYQNALSNFSGKK	CT045	Leucyl aminopeptidase	PepA
GPKGRHVVIDKSFGSPQVTKDGVT	CT110	Chaperonin GroEL1	GroEL1
EERVVGQPFAIAAVSDS	CT113	Clp Protease ATPase	ClpB
DLKVTGPTIHTDLD	CT143	Hypothetical protein CT143	CT143
GKLIVTNPKSDISFGG	CT144	Hypothetical protein CT144	CT144
GSPGQTNYAAAKAGIIGFS	CT237	3-ketoacyl-(acyl-carrier-protein) reductase	FabG
GTKTPIGTPIAVFSTEQ	CT247	Dihydrolipoamide acetyltransferase	PdhC
SPKEAAIAAARASLSPEEKR	CT289	Hypothetical protein CT289	CT289
YDHIIVTPGANADILPE	CT375	Predicted D-Amino Acid Dehydrogenase	
FDGEKASVGAPTVGNAVVKG	CT420	50S ribosomal protein L21	Rl21
KLDGVSSPAVQESISESL	CT424	Sigma Regulatory factor	RsbV
TPSAVNPLPNPEIDS	CT472	Hypothetical protein CT472	CT472
DSTHGSFAPQATFSDG	CT505	Glyceraldehyde-3-phosphate dehydrogenase	GapA
VKGNEVFVTPAAHVVDRPG	CT514	50S ribosomal protein L6	RplF
KPAPKETPGAAEGAEAQTASEQPSKEN AEKQEENNEDA (one Peptide)	CT559	Yop proteins translocation lipoprotein	CdsJ
ADVLLLSPKASVSPGG	CT561	Type III secretion translocase	CdsL
IPFAKPDANLSAED	CT619	Hypothetical protein CT619	CT619
KAPQFGYPAVQNSADS	CT622	CHLPN 76kDa Homolog	
KEGEEDTAESAANEEPKAEASQEEE	CT664	FHA domain; homology to adenylate cyclase	
IFDTTTLNPTIAGAGDVK	CT681	Major Outer Membrane Protein	MOMP
TPVESTTPVAPEISVVNAK	CT759	Muramidase (invasin repeat family)	NlpD
QVFQLITQVTGRSG	CT778	Primosome assembly protein	PriA
ISYDYSSGNAEASSHN	CT837	Hypothetical protein CT837	CT837
DAGVPIKAPVAGIAMG	CT842	Polyribonucleotide Nucleotidyltransferase	Pnp
GSVVFSGATVNSADFH	CT869	Polymorphic membrane protein E	PmpE
AMANEAPIAFIANVAG	CT871	Polymorphic membrane protein G	PmpG
AEKGGGAIYAPTIDISTNGGS	CT872	Polymorphic membrane protein H	PmpH

Table III: Ten overlapping source proteins between *C. trachomatis* and *C. muridarum*. Identical amino acid residues of the epitopes between the two strains are underlined and in bold. Percentage protein identity is for the whole length proteins of each comparison.

C.trachomatis **Locus# / Protein abbreviation**	***C.trachomatis*-derived Peptides**	***C. muridarum*** **Locus#**	***C.muridarum*-derived Peptides**	***C. trachomatis / C. muridarum*** **Protein Identity**
CT143	**DL**K**VTGP**T**I**H**TD**L**D**	TC0420	**DL**N**VTGP**K**I**Q**TD**V**D**	75%
CT237 (FabG)	G**SPGQTNYAAAKAGIIGFS**	TC0508	**SPGQTNYAAAKAGIIGFS**	90%
CT247 (PdhC)	**GTK**T**PIGTPIAVFSTEQ**	TC0518	E**GTK**I**PIGTPIAVFSTEQ**N	87%
CT424 (RsbV)	**KLDGVSSPAVQESISE**SL	TC0707	**KLDGVSSPAVQESISE**	96%
CT375	**YDHIIVTPGANADIL**PE	TC0654	**YDHIIVTPGANADIL**	85%
CT505 (GapA)	DSTHGSFAPQATFSDG	TC0792	MTTVHAATATQSVVD	95%
CT514 (RplF)	**VKGNEVFV**T**PAAH**VV**DRPG**	TC0801	**VKGNEVFV**S**PAAH**II**DRPG**	96%
CT869 (PmpE)	GSVVFSGATVNSADFH	TC0261	SRALYAQPMLAISEA	69%
CT871 (PmpG)	AMANEAPIAFIANVAG	TC0263	NAKTVFLSNVASPIYVDPA ASPIYVDPAAAGGQPPA	71%
CT872 (PmpH)	AEKGGGAIYAPTIDISTNGGS	TC0264	SPQVLTPNVIIPFKGDD	76%

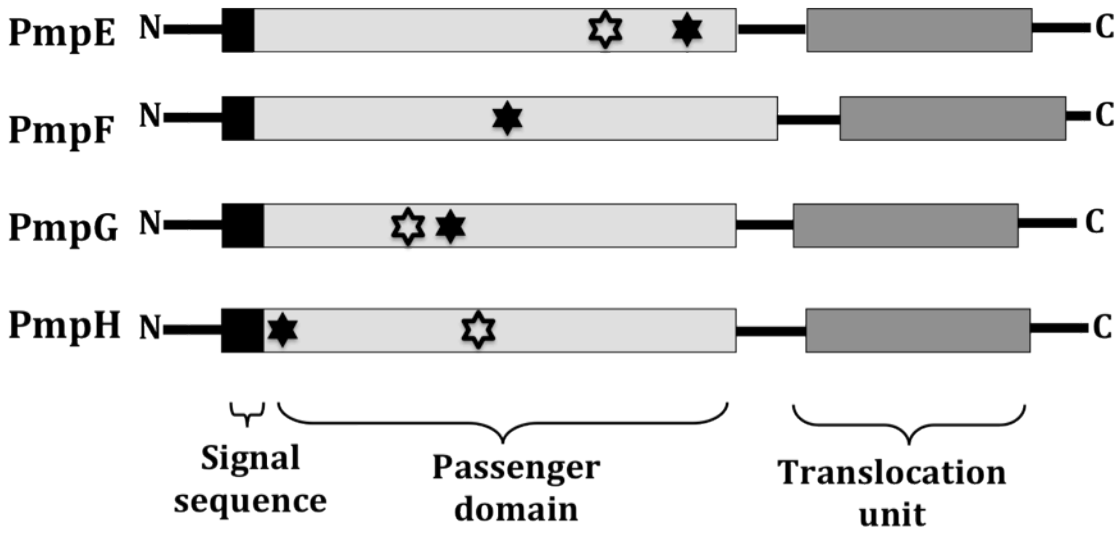

Figure 1: Location of MHC class II-bound peptides derived from *C. muridarum* (filled star) and *C. trachomatis* (open star) within the predicted passenger domains of the Pmp proteins.

C. trachomatis (CT681)	I**FDT**TT**L**NPTI**A**G**A**G**DVK**
C. muridarum (TC052)	I**LXM**TT**W**NPTI**S**G**S**G---
	*** * * * ***

Figure 2: Alignment of MHC-bound peptide derived from *C. trachomatis* major outer membrane protein (CT681) in relation to *C. muridarum* homolog (TC052). The non-conserved residues are in bold and marked by asterisks.

REFERENCES

1. **Brunham, R. C., B. Pourbohloul, S. Mak, R. White, and M. L. Rekart. 2005. The unexpected impact of a Chlamydia trachomatis infection control program on susceptibility to reinfection. *J Infect Dis* 192:1836-1844.**
2. **Grayston, J. T., and S. P. Wang. 1978. The potential for vaccine against infection of the genital tract with Chlamydia trachomatis. *Sex Transm Dis* 5:73-77.**
3. **Morrison, R. P., and H. D. Caldwell. 2002. Immunity to murine chlamydial genital infection. *Infect Immun* 70:2741-2751.**
4. **Steinman, R. M., and M. Pope. 2002. Exploiting dendritic cells to improve vaccine efficacy. *J Clin Invest* 109:1519-1526.**
5. **Read, T. D., R. C. Brunham, C. Shen, S. R. Gill, J. F. Heidelberg, O. White, E. K. Hickey, J. Peterson, T. Utterback, K. Berry, S. Bass, K. Linher, J. Weidman, H. Khouri, B. Craven, C. Bowman, R. Dodson, M. Gwinn, W. Nelson, R. DeBoy, J. Kolonay, G. McClarty, S. L. Salzberg, J. Eisen, and C. M. Fraser. 2000. Genome sequences of Chlamydia trachomatis MoPn and Chlamydia pneumoniae AR39. *Nucleic Acids Res* 28:1397-1406.**
6. **Stephens, R. S., S. Kalman, C. Lammel, J. Fan, R. Marathe, L. Aravind, W. Mitchell, L. Olinger, R. L. Tatusov, Q. Zhao, E. V. Koonin, and R. W. Davis. 1998. Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. *Science* 282:754-759.**
7. **de Jong, A. 1998. Contribution of mass spectrometry to contemporary immunology. *Mass Spectrom Rev* 17:311-335.**
8. **Karunakaran, K. P., J. Rey-Ladino, N. Stoynov, K. Berg, C. Shen, X. Jiang, B. R. Gabel, H. Yu, L. J. Foster, and R. C. Brunham. 2008. Immunoproteomic discovery of novel T cell antigens from the obligate intracellular pathogen Chlamydia. *J Immunol* 180:2459-2465.**
9. **Yu, H., K. P. Karunakaran, I. Kelly, C. Shen, X. Jiang, L. J. Foster, and R. C. Brunham. Immunization with live and dead Chlamydia muridarum induces different levels of protective immunity in a murine genital tract model: correlation with MHC class II peptide presentation and multifunctional Th1 cells. *J Immunol* 186:3615-3621.**
10. **Yu, H., X. Jiang, C. Shen, K. P. Karunakaran, and R. C. Brunham. 2009. Novel Chlamydia muridarum T cell antigens induce protective immunity against lung and genital tract infection in murine models. *J Immunol* 182:1602-1608.**
11. **Yu, H., X. Jiang, C. Shen, K. P. Karunakaran, J. Jiang, N. L. Rosin, and R. C. Brunham. Chlamydia muridarum T-cell antigens formulated with the adjuvant DDA/TDB induce immunity against infection that correlates with a high frequency of gamma interferon (IFN-gamma)/tumor necrosis factor alpha and IFN-gamma/interleukin-17 double-positive CD4+ T cells. *Infect Immun* 78:2272-2282.**

12. **Caldwell, H. D., J. Kromhout, and J. Schachter. 1981. Purification and partial characterization of the major outer membrane protein of Chlamydia trachomatis. *Infect Immun* 31:1161-1176.**
13. **Su, H., R. Messer, W. Whitmire, E. Fischer, J. C. Portis, and H. D. Caldwell. 1998. Vaccination against chlamydial genital tract infection after immunization with dendritic cells pulsed ex vivo with nonviable Chlamydiae. *J Exp Med* 188:809-818.**
14. **Cox, A., Huczko, E. L., Engelhard, V. H., Shabanowitz, J., *et al*. 1997. The application of mass spectrometry to the analysis of peptides bound to MHC molecules. In *MHC - A practical Approach*. a. G. B. N. Fernandez, ed. 142-160.**
15. **Ishihama, Y., J. Rappsilber, and M. Mann. 2006. Modular stop and go extraction tips with stacked disks for parallel and multidimensional Peptide fractionation in proteomics. *J Proteome Res* 5:988-994.**
16. **Hsu, J. L., S. Y. Huang, N. H. Chow, and S. H. Chen. 2003. Stable-isotope dimethyl labeling for quantitative proteomics. *Anal Chem* 75:6843-6852.**
17. **Kari, L., W. M. Whitmire, N. Olivares-Zavaleta, M. M. Goheen, L. D. Taylor, J. H. Carlson, G. L. Sturdevant, C. Lu, L. E. Bakios, L. B. Randall, M. J. Parnell, G. Zhong, and H. D. Caldwell. A live-attenuated chlamydial vaccine protects against trachoma in nonhuman primates. *J Exp Med* 208:2217-2223.**
18. **Brunham, R. C., and J. Rey-Ladino. 2005. Immunology of Chlamydia infection: implications for a Chlamydia trachomatis vaccine. *Nat Rev Immunol* 5:149-161.**
19. **Suri, A., S. B. Lovitch, and E. R. Unanue. 2006. The wide diversity and complexity of peptides bound to class II MHC molecules. *Curr Opin Immunol* 18:70-77.**
20. **Sette, A., and B. Peters. 2006. Immune epitope mapping in the post-genomic era: lessons for vaccine development. *Curr Opin Immunol*.**
21. **Schautteet, K., E. De Clercq, and D. Vanrompay. Chlamydia trachomatis vaccine research through the years. *Infect Dis Obstet Gynecol* 2011:963513.**
22. **Su, H., R. P. Morrison, N. G. Watkins, and H. D. Caldwell. 1990. Identification and characterization of T helper cell epitopes of the major outer membrane protein of Chlamydia trachomatis. *J Exp Med* 172:203-212.**
23. **Ortiz, L., K. P. Demick, J. W. Petersen, M. Polka, R. A. Rudersdorf, B. Van der Pol, R. Jones, M. Angevine, and R. DeMars. 1996. Chlamydia trachomatis major outer membrane protein (MOMP) epitopes that activate HLA class II-restricted T cells from infected humans. *J Immunol* 157:4554-4567.**
24. **Brunham, R. C., and R. W. Peeling. 1994. Chlamydia trachomatis antigens: role in immunity and pathogenesis. *Infect Agents Dis* 3:218-233.**

25. Cohen, C. R., K. M. Koochesfahani, A. S. Meier, C. Shen, K. Karunakaran, B. Ondondo, T. Kinyari, N. R. Mugo, R. Nguti, and R. C. Brunham. 2005. Immunoepidemiologic profile of Chlamydia trachomatis infection: importance of heat-shock protein 60 and interferon- gamma. *J Infect Dis* 192:591-599.

26. Bavoil, P. M., Wyrick, P.B. 2006. *Chlamydia: Genomics and Pathogenesis*. Horizon Bioscience.

27. Saka, H. A., J. W. Thompson, Y. S. Chen, Y. Kumar, L. G. Dubois, M. A. Moseley, and R. H. Valdivia. Quantitative proteomics reveals metabolic and pathogenic properties of Chlamydia trachomatis developmental forms. *Mol Microbiol*.

28. Giles, D. K., and P. B. Wyrick. 2008. Trafficking of chlamydial antigens to the endoplasmic reticulum of infected epithelial cells. *Microbes Infect* 10:1494-1503.

29. Giles, D. K., J. D. Whittimore, R. W. LaRue, J. E. Raulston, and P. B. Wyrick. 2006. Ultrastructural analysis of chlamydial antigen-containing vesicles everting from the Chlamydia trachomatis inclusion. *Microbes Infect* 8:1579-1591.