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Structural Modelling of the Chlamydia Trachomatis Major Outer Membrane Protein Provides Insights into Immunogenic Properties of its External loops in Serotypes E and K

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Abstract. Chlamydia trachomatis is one of the most prevalent sexually transmitted pathogens, causing serious health risks worldwide. Based on serological properties of the chlamydial major outer membrane protein, up to 19 serovars of C.trachomatis can be distinguished, having different antigenic properties and clinical manifestations. In this study, the antibody response to the variable domains of the major outer membrane protein of *C. trachomatis* serotypes E and K was analyzed in connection with the structural models of its extracellular loops. We propose that the reduction of antibody formation to the antigens of some variable domains may be caused by their shielding by other external loops. It has been shown that the entire structure of the second and fifth loops of genotype E are more compact and have minimal chain exposure due shielding with the more unfolded third and seventh loops. This corresponds to the absence of significant antibody response to their variable domains VDI and VDII. In genotype K entire structure of the second and the fifth loops is more unfolded, their shielding with the third and seventh loops is not so complete. As a consequence their variable domains induce antibody production in infected organism.

Keywords: *Chlamydia trachomatis*; serotypes E, K, major outer membrane protein; variable domains; antibody formation; models of external loops; shielding.

Introduction. The major outer membrane protein (MOMP) of *Chlamydia trachomatis* is its most prevalent and immunodominant outer membrane protein, making up to 60% of total membrane protein content. MOMP belongs to the -barrel family, has 16 transmembrane -strands and 8 extracellular loops. The main linear B-cellular epitopes of MOMP are located in 4 extracellularly exposed variable domains. The localization of the epitopes in variable domains is well studied and reviewed elsewhere [1, 2]. Based on the antigenic variability of the MOMP, more than 17 serotypes of *C. trachomatis* can be distinguished, differing by antigenic properties and, to some extent, by clinical manifestations of the infection.

Unfortunately, structural properties of the extracellular loops, which determine the strength of antigenic response to the MOMP, have not been studied in sufficient details. Based on this, we modelled extracellular loops of the serotypes E and K and correlated their properties with the strength of the antigenic response to their variable domains. Our results shed light on potential mechanisms of mutual domain shielding and on the extent of their extracellular exposure.

Materials and methods. The models were built according to Wang et al. [3] (Fig.1).

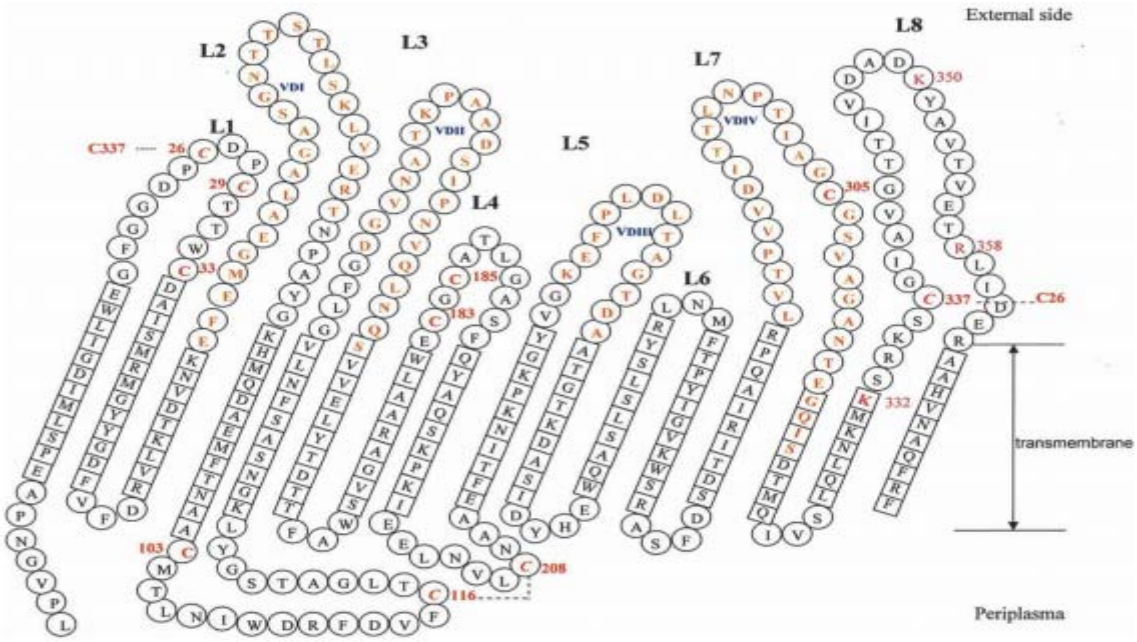

Fig. 1. Topology sketch of MOMP as viewed from the barrel exterior in accordance with Wang et al. [3].

Homology modelling of the MOMP molecule was performed using the SWISS-MODEL server [4], based on the *E.coli* OmpF structure (protein data bank accession number 2OMF, chain A). Sequence alignment and determination of homologous regions were performed by the Geneious Pro 5.6.5 software. Extracellular loops were modelled using the PEP-FOLD server [5, 6]. Determination of the antigenic epitopes on the extracellular loops was done by the SVMTrip [7].

The data about antigenicity of the MOMP variable domains and B-cellular epitope localization were based on Nunes` et al. review data, summarized in supplementum [1]. The strength of the antigenic response to different variable domains was estimated based on the number of publications, which detected antibodies against different MOMP peptides, published by Nunes et al [1]. Estimation of the hydrophilicity was done using sum of Hopp and Woods coefficients for all amino acids of corresponding external loops of the MOMP [8].

Results and discussion. From 8 external loops of the MOMP only four – L2, L3, L5 and L7 - contain variable domains VDI, VDII, VDIII and VDIV with antigenic epitops for B-cells. The longest eight loop is connected by disulphide bond with the first loop and folds into the barrel channel. The fourth and sixth loops are too small to induce significant antibody response.

The summarized data about the strength of the antigenic response to the MOMP variable domains, as well as their size and the hydrophilicity of the corresponding extracellular loops of serotypes E and K are shown in the Table. They suggest significant differences in the intensities of antibody production in response to the variable domains of the *C. trachomatis* MOMP. The differences become particularly striking taking into the account differences of the domain sizes and corresponding extracellular loops shown in the table

.The strongest immune response was induced by the antigens of the 4th variable domain, which is the longest among the variable domains of MOMP. The first variable domain induces significantly lower antigenic response, despite its relatively large size. It could be suggested that the first domain has not active antigenic epitopes, but the SVMTrip server predicts quite strong epitopes in this region. High hydrophilicity of the first loop also suggests high immunogenicity of the first variable domain [8]. The discrepancy between size and hydrophilicity, on the one side, and immunogenicity, on the other side, can be explained by shielding of the first domain by other structures. To verify this suggestion, we analyzed structures of MOMP and its extracellular loops by *in silico* modelling.

Table

Some properties of the variable domains and extracellular loops of MOMP in *C. trachomatis* serotypes E and K

Property	Serotype	VDI	VDII	VDIII	VDIV
Antibody production	E	0	3	0	13
	K	2	2	2	9
Size of domains, amino acids	E	20	21	12	32
	K	22	22	12	33
Size of extracellular loops, amino acids	E	30	25	16	32
	K	32	26	16	33
Hydrophilicity of loops	E	7,7	3,2	- 5,8	1,8
	K	6,3	0,2	- 6,7	0,8

Fig.2 shows a model of the *C. trachomatis* serotype E MOMP. This model suggests that overlapping extracellular loops can significantly shield each other and mask antigenic determinants. It can be proposed that the second loop is mostly shielded by the neighboring third extracellular loop, which has a size of 25-26 amino acids. The structure of these loops can be of particular importance. Comparision of the models of the four extracellular loops containing variable domains of serotypes E and K (Fig. 3-6) can explain some features of antigenic response to the MOMP antigens of these serotypes.

The second loop contains two constant 5 amino acid long chains, flanking the 20-amino acid long variable domain (Fig. 3).

Figure 2. A model of the MOMP molecule. Shielding of some extracellular loops by the other can be observed

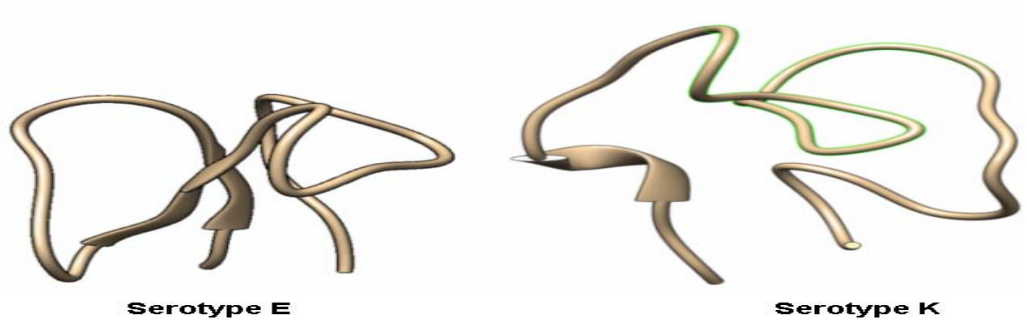

Figure 3. Comparison of second extracellular loops between genotypes E and K. The antibody-binding region is highlighted in green

These chains correspond to vertical fragments of the terminal sites of the serotype E loop. Constant structure of this fragments suggests that they are not externally exposed due to the shielding by the surrounding chains.

Comparison of the structures of the second loop in genotypes E and K shows that genotype E has minimal chain exposure, significant parts of it are hidden, and the entire structure is quite compact. Presumably, this ensures significant shielding of the first variable domain of serotype E by the neighboring third loop. The structure of the first domain of genotype K is more unfolded and elevated, making its better exposure and explaining higher antigenicity of its central part (highlighted in green).

To some extent, different levels of shielding of the first domain in genotypes E and K can be explained by structural features of the third loop, shown in Fig. 4.

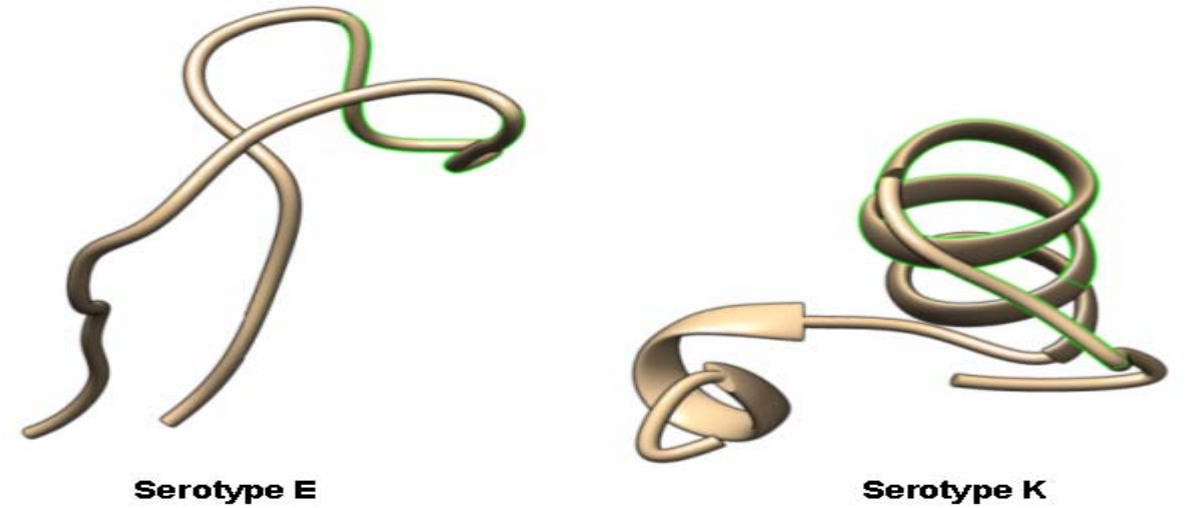

Figure 4. Comparison of the third extracellular loop in genotypes E and K. The antibody-binding region is highlighted in green.

In serotype E the third loop is more elevated, whereas in serotype K it is more compact and folded. Accordingly to this, a shielding of the first domain of the second loop by the third loop will be more complete in genotype E. Moreover, the elevated third loop of serotype E induces stronger antibody production than in serotype K. The models of the fifth extracellular loop are shown in Fig. 5. The third variable domain is relatively small In size.

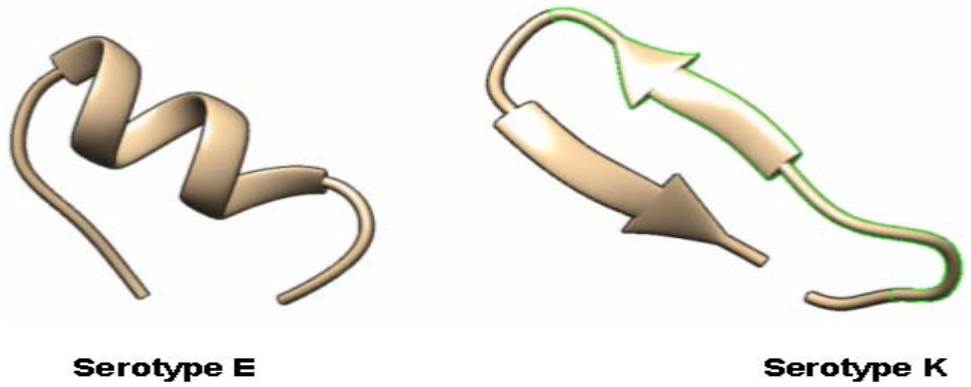

Figure 5. Comparison of the fifth extracellular loop in genotypes E and K. The antibody-binding region is highlighted in green.

Despite its significant similarities in genotypes E and K, this loop in genotype K is less compact and stimulates antibody production, unlike to genotype E. The large seventh loop with the fourth variable domain also influences immunogenicity of the fifth loop (Fig. 6). In genotype E it is more elevated and oriented towards the third variable domain of the fifth loop. This ensures better

shielding of the neighboring third variable domain. The seventh loop of serotype K forms circles in its lower part, which reduces the height of the loop. Besides that, it is oriented to the opposite direction to the third variable domain, reducing its shielding and promoting the antibody production against its epitope.

In **conclusion** it should be noted, that comparative analysis of the structural models of the extracellular loops of the MOMP with their variable domains, explains some features of the antibody production in different serotypes of Chlamydia. It can be also suggested that serotype E has some peculiarities in the MOMP presentation, which can provide this serotype with additional advantages in adaptation to the defense mechanisms of the host. It is known that this serotype is the most prevalent in many regions of the world and has low mutation frequency in the MOMP gene [2. 9, 10]. It can be partly explained by the localization of its MOMP antigenic epitopes only in two

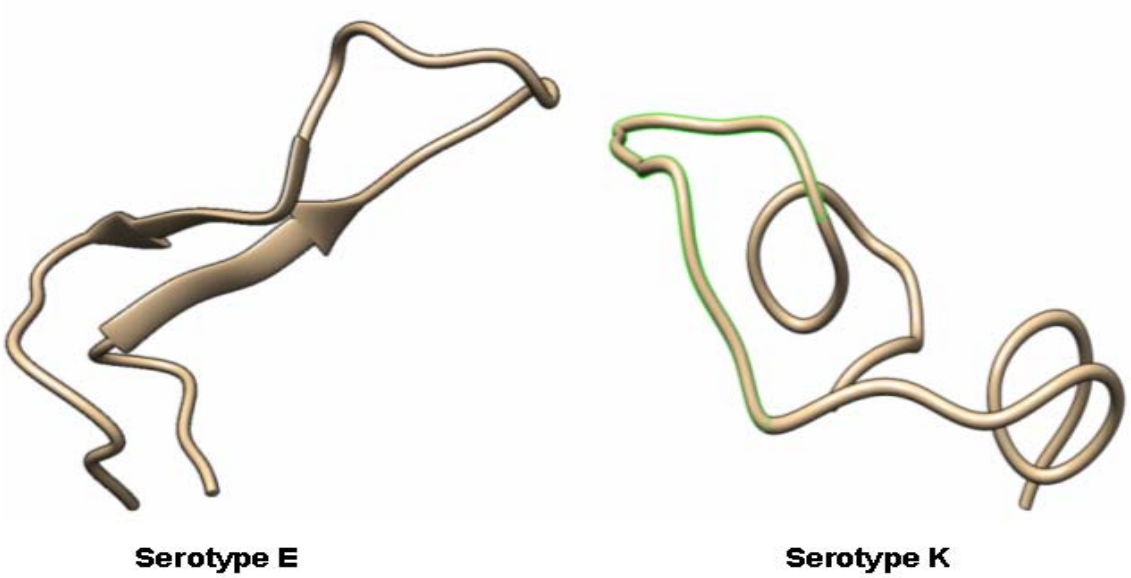

Figure 6. Comparison of the seventh extracellular loop in genotypes E and K. The antibody-binding region of serotype K is highlighted in green, in sepotype E antibodies bind to almost all parts of the loop

variable domains. This epitope localization can reduce damaging effect of the host immune system and protect *C. trachomatis* due to the more narrow range of its antigenic epitopes.

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Структура внеклеточных петель главного белка наружной мембраны серотипов Е и К Chlamydia trachomatis и их способность индуцировать образование антител

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Аннотация. *Chlamydia trachomatis* принадлежит к наиболее распространенным возбудителем инфекционных заболеваний человека. На основании антигенных свойств главного белка наружной мембраны хламидий выделяются более 19 сероваров хламидий, отличающихся по свои антигенным характеристикам и клиническим проявлениям заболеваний. В настоящем исследовании антительный ответ на вариабельные домен главного белка наружной мембраны *C. trachomatis* серотипов Е и К сопоставлялся с структурными моделями их внеклеточных петель. Было показано, что структура второй и пятой петель генотипа Е более компактна и характеризуется минимальной экспзицией вследствие экранирования более развернутыми третьей и седьмой петлями. Это соответствует отсутствию заметного образования антител на их вариабельные домены VDI и VDIII. У серотипа К структура второй и пятой петель более развернута, они не полностью экранируются третьей и седьмой цепями. Это соответствует образованию антител на их вариабельные домены в инфицированном организме.

Ключевые слова: Chlamydia trachomatis; серотипы Е и К; главный белок наружной мембраны; вариабельные домены; образование антител; модели внеклеточных петель; экранирование.