

## Lack of Allelic Polymorphism for the Major Outer Membrane Protein Gene of the Agent of Guinea Pig Inclusion Conjunctivitis (*Chlamydia psittaci*)

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**The major outer membrane protein gene (*omp1*) was sequenced for each of six *Chlamydia psittaci* (guinea pig inclusion conjunctivitis [GPIC]) strains isolated from guinea pigs. Five of the isolates were obtained in the United States during the 1960s and 1970s, including the prototype strain isolated by Murray in 1962. The other isolate was obtained from a guinea pig in England. The nucleotide sequence of the *omp1* gene for each strain was identical. The lack of *omp1* allelic polymorphism among GPIC isolates suggests that, unlike *C. trachomatis*, the GPIC agent lacks antigenic variation in the major outer membrane protein.**

*Chlamydia trachomatis* organisms are obligate intracellular bacteria that cause of a wide spectrum of human ocular and genital tract diseases. Efforts to characterize the immunobiology of *C. trachomatis* have involved study of chlamydial antigens and immune responses, using animal models. However, immunity to chlamydial infection remains poorly understood. *C. trachomatis* is composed of 15 serovariants that can be discriminated by using specific monoclonal antibodies (18). The serovariant antigen of *C. trachomatis* is the major outer membrane protein (MOMP). The MOMP is antigenically complex, displaying a mosaic of antigens with different specificities (18), and sequencing of the *C. trachomatis* MOMP genes (*omp1*) of different serovars reveals sequence polymorphism in four distinct regions (17, 22). Monoclonal antibodies of different specificities have been mapped to three of these regions (1, 19).

On the basis of human vaccine trials and nonhuman primate models, serovar-specific antigens have been associated with protective immune responses (15). Thus, it is thought that anti-MOMP antibodies play important functional roles in protective immune responses and that MOMP antigens should be good vaccine candidates. In support of a protective function for anti-MOMP antibodies, monoclonal antibodies to serovar-specific antigens neutralize chlamydial infectivity in vitro (20) and when coinoculated into the eyes of monkeys (24). Species-specific antibodies are also capable of neutralizing infectivity in vitro, although perhaps less efficiently (8).

Since the isolation of a chlamydial agent that naturally infects guinea pigs, called the guinea pig inclusion conjunctivitis (GPIC) agent (7), the guinea pig has served as an important small animal model to investigate immune responses to chlamydiae. However, the GPIC agent is a different chlamydial species, *C. psittaci*, than the natural human pathogen, *C. trachomatis*. Immune protection has been demonstrated in guinea pig models of ocular and genital tract infection. T-cell responses are important in immune protection in the GPIC models (5, 10, 13), and antibody responses have also been associated with protection against

infectious challenge and with clearance of infection (6, 11, 12, 14).

The usefulness of the GPIC model as a correlate to human *C. trachomatis* infection would be greatly expanded if antigenically distinct strains of GPIC could be used in inoculation and challenge experiments. This would more directly define the role of serovar-specific humoral immune responses in immune protection and could be used to precisely define the antigenic moieties on MOMP that are important for immune protection. We evaluated a number of previously isolated GPIC strains by nucleotide sequencing of the GPIC *omp1* gene to identify strains that, by virtue of variant nucleotide sequences, could be tested for fine differences in antigenic character. However, *omp1* sequencing of six strains isolated in different years and from different continents demonstrated that all had identical nucleotide sequences. Thus, no evidence of MOMP-based antigenic differences among GPIC strains was found.

Three GPIC strains were obtained from stocks at the UCSF Chlamydia Laboratory. Each was originally isolated from a guinea pig that was from a commercial supplier. One, GP-86, was isolated in 1964 and has been used for most laboratory and animal model studies of GPIC conducted at the University of California, San Francisco. Two others, GP-3 and GP-14, were isolated in 1966 and 1977, respectively. Two strains, 62H464 and 73H301, were generously provided by R. Rank, University of Arkansas, although they were isolated by Murray (7). 73H301 was a passage dating from a 1973 isolation. 62H464 is an original pool of GPIC organisms isolated by Murray in 1962 (7). This isolate was in egg amniotic fluid from which a few microliters were chipped from the frozen specimen and used for DNA amplification. J. Treharne, University of London, generously provided a sample of IOL-1012, which he originally isolated in London from a Dunkin Hartley guinea pig in 1975.

DNA from GPIC was isolated by lysis of the organisms in 50 mM dithiothreitol with boiling, and the DNA was purified by using GeneClean (Bio 101, La Jolla, Calif.). Four oligonucleotides were used as primers to amplify *omp1* by polymerase chain reaction (PCR). The primers were designed with 5' *Bam*HI and 3' *Eco*RI restriction endonuclease sites for directional cloning into plasmid pUC19. The sequences

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of the primers are as follows: (i) 5'-AAAAGAATTCACAT TCCCATAAAGCTCCACG-3'; (ii) 5'-AAAAGGATCCTAC GGAGATTATGTTTTTCGAT-3'; (iii) 5'-AAAAGGATCCC GTGGAGCTTTATGGGAATGT-3'; (iv) 5'-AAAAAATTC ACCACAAGCTTTTCTAGACTT-3'. Primer pairs i and ii were used to amplify the VS1/VS2 region (nucleotides 193 to 598), and primers iii and iv were used to amplify the VS3/VS4 region (nucleotides 577 to 1062). Two microliters of purified DNA was used as the template, and PCR was performed following the manufacturer's instructions (Gene Amp; Perkin-Elmer Cetus, Norwalk, Conn.).

The amplified gene products were cleaved with *Bam*HI and *Eco*RI, and the restriction fragments were ligated into pUC19 and used to transform *Escherichia coli* TB1. Preparations of plasmid DNA from the ampicillin-resistant colonies were obtained by the alkaline lysis method. The *omp1* gene fragments were sequenced by using an M13 dye primer (Taq Dye Primer; Applied Biosystems, Burlingame, Calif.), and the sequence was analyzed on an Applied Biosystems Model 370A DNA sequencer. PCR, cloning, and sequencing were conducted to completion using only one strain at a time, and PCR products for IOL-1012 were obtained at a different laboratory site with separate reagents.

Using PCR amplification of the GPIC *omp1* gene, we determined over 70% of the *omp1* nucleotide sequence for each of six independently isolated GPIC strains. We found no variation in the nucleotide sequences among these strains, and each sequence was the same as previously reported (23). The results of this survey of GPIC strains suggest that, unlike *C. trachomatis* strains, variation in MOMP sequence and related variations in surface antigens are not properties of the GPIC agent.

It has been proposed that *C. trachomatis* antigenic differences represented by allelic antigenic variation of the MOMP gene among the population of *C. trachomatis* strains result from selective pressure exerted by host immune responses (16). If this is the mechanism that drives variation in MOMP antigens, then MOMP antigens must be effective targets for the host immune response and sufficiently important for the immunobiology of *C. trachomatis* that these phenotypes have been selected.

The lack of MOMP antigenic heterogeneity among GPIC strains represents an important limitation for the guinea pig model in evaluation of the role of antigenic variation in immune protection. Apparently the GPIC agent is under different selective pressures than human strains of *C. trachomatis*. This could be because there are fundamentally different immune mechanisms that control GPIC infection or an antigen other than MOMP is the target of immune protection. An alternative cause for this difference could be rooted in some basic biological differences in pathogenic potential in the context of the host-parasite interaction. Such factors that could mitigate the need for *omp1* allelic polymorphism because of a change in immune selective pressure include the duration of infection, frequency of reinfection, longevity and intensity of effective host immune responses, and the length of time the immune response has access to the organisms.

Unfortunately the pedigree of each of the isolates studied cannot be reconstructed with confidence. Nevertheless, if one were to consider that these isolates all represented a common source of contamination that has been propagated in captive guinea pig colonies for many years, it remains remarkable that variation was not observed since the selective pressure would likely be even higher than in wild populations. These findings do not change the possibility of

finding variant GPIC strains in the wild; however, the temporal and geographical diversity of the collection tested suggests that variation is not common and not represented among the strains currently or historically used for laboratory experimentation.

Recently, *C. pneumoniae* has been implicated as a common cause of epidemic human pneumonitis. Currently, the information available, although limited, suggests that for *C. pneumoniae* there is not a prominent anti-MOMP serological response to denatured MOMP compared with its *C. trachomatis* counterparts (3). This is similar in some respects to the serological response observed for GPIC infections (2, 21). Gene sequence comparisons for two different *C. pneumoniae* strains do not show *omp1* polymorphism (4, 9). If *C. pneumoniae omp1* is not sequence variant, then understanding the mechanism for selective adaptations of *C. trachomatis* and *C. pneumoniae* in the context of the immunobiology of chlamydiae will be important for developing appropriate immune intervention strategies.

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