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 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 serovars 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 coinfected 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 generated 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 of 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 Gene-clean (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' BamHI and 3' EcoRI restriction endonuclease sites for directional cloning into plasmid pUC19. The sequences

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of the primers are as follows: (i) 5′-AAAAGAATTACAT
TCCATATAAACCCACG-3′; (ii) 5′-AAAAAGATCTTAC
GGAGATGTTTTGCAG-3′; (iii) 5′-AAAAAGATCCC
GGGAGTTTTGAGAATGTT-3′; (iv) 5′-AAAAATTC
ACCACAGCTTTCAGACT-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
Ampl; Perkin-Elmer Cetus, Norwalk, Conn.).

The amplified gene products were cleaved with BamHI
and EcoRI, and the restriction fragments were ligated into
pUC19 and used to transform Escherichia coli TB1. Prepa-
rations of plasmid DNA from the ampicillin-resistant colo-
nies were obtained by the alkaline lysis method. The ompl
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 ompl 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 differ-
ences 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 impor-
tant 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. trach-
omatis. 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 poly-
orphism 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
were one 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 selec-
tive 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 labora-
tory experimentation.

Recently, C. pneumoniae has been implicated as a com-
mon 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. trach-
omatis 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. pneu-
moniae strains do not show ompl polymorphism (4, 9). If C.
pneumoniae ompl is not sequence variant, then understand-
ing the mechanism for selective adaptations of C. tracho-
matis and C. pneumoniae in the context of the immunobi-
ology of chlamydiae will be important for developing
appropriate immunization strategies.

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