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**Development of Specific Assays for Diagnosis of *Chlamydia trachomatis*
and TWAR (*C. pneumoniae*) Infections**

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EXECUTIVE SUMMARY

Chlamydia are intracellular bacterial pathogens which cause a variety of serious diseases in the eye and in the genito-urinary and respiratory tracts, and may be responsible for some heart disease and certain forms of arthritis. The purpose of the project "Development of specific assays for diagnosis of *Chlamydia trachomatis* and TWAR (*Chlamydia pneumoniae*) infections" is to develop both serologic and agent detection assays which will be capable of distinguishing between infections with these two organisms which have some genus-specific (common) attributes and some species-specific attributes. During the first year of this grant, collection of paired serum samples and throat swabs from patients with community acquired pneumonia (CAP) in Israel (n=330) was completed. In parallel, serum samples and cervical swabs were collected from women with suspected pelvic inflammatory disease (PID) (n=175) and with cervicitis (n=101). These samples were tested by accepted techniques for evidence of chlamydial infection and were used to calibrate the new techniques which we are developing in our laboratories. We have now completed development of an ELISA technique for differentiation of antibodies produced against the two pathogens and have used it to test the close to 300 serum samples collected from children with respiratory infections or healthy controls in Peru and Bolivia. Our Peruvian collaborators have also collected serum, urine, and cervical samples from women at high altitudes and from women attending gynecologic clinics in Lima. As a result of three productive exchange visits, Chlamydia detection methods, based on specific PCR genome detection which we developed, are now in use in Peru, as is the ELISA serologic technique. Implementation of the new techniques has expanded the capabilities of our collaborators' laboratory, and application of the methods to samples obtained in Peru will help determine the extent of illness caused by both respiratory and sexually transmitted chlamydial infections, hopefully leading to effective interventions and improvement in the health of various communities in this developing region.

Section I

A. Research Objectives

The primary objective of this research project is to develop techniques for differentiation of infection with *Chlamydia trachomatis* from infection with *Chlamydia pneumoniae*. This involves development of serologic assays which will be able to identify the presence of antibodies against each of these two species and development of direct detection techniques that will be capable of detecting these organisms in appropriate clinical specimens. In order to accomplish these objectives, we have collected paired serum samples and throat swabs from 330 patients hospitalized with community acquired pneumonia (CAP) as well as paired serum samples and cervical or urethral samples from 175 patients with pelvic inflammatory disease (PID), and 101 with vaginitis/cervicitis. In Peru samples have been collected from healthy children and adults in the Pampas (San Juan--84 serum samples) and from children with acute respiratory disease or pneumoniae (90 acute and 74 convalescence sera; 25 nasopharyngeal lavages; and 55 boiled urine samples for PCR). In addition sera, urine, and cervical samples were collected from women attending gynecologic clinics and residing at high altitudes; cervical swabs and urine samples were collected from women attending family planning clinics; and 84 serum samples were collected from healthy women in Bolivia. An ELISA test capable of distinguishing between antibodies to the two organisms has been developed and only minimal refinement is required. This will be done during the coming year. The PCR assay for detection of chlamydia in clinical specimens has been developed. During the coming year its sensitivity for detection of organisms in clinical samples will be further assessed, and the final signal detection method established.

B. Research Accomplishments

1. Development of serologic tests and results obtained with them

We have now completed development of an ELISA technique which is able to indicate the chlamydial species against which most reactive antibodies in the serum are directed. When the technique is used for detection of serum IgG alone, it indicates the serologic status of the subject vis a vis past infection with each of the two chlamydial species. When used in conjunction with IgA detection, it seems capable of indicating current infection as well. We are now convinced that it will not be able to be patented, however, because while a similar technique has not been used for this purpose, 6M urea has been used to reduce non-specific binding in certain ELISA type assays. We have submitted

our first manuscript on the technique for publication and anticipate submitting a number of additional manuscripts during the coming year.

Preliminary analysis of the results obtained in testing the sera collected from the various groups in Peru indicates that *C. trachomatis* positivity ranges from 35% in the San Juan poor of Lima (n=84) and a Bolivian sample of healthy women (n=84), to 45% in a sample of high altitude women (n=58), but 0% in a group of children (aged 0-10 years, n=88) with severe respiratory infection in Lima. In these same groups seropositivity to *C. pneumoniae* was 65%, 80%, and 25%, respectively. For the children, reference is to acute phase serum samples. In the age group 2-5 years (n=28), 35% were seropositive and in the age group 5-10 years (n=17), 50% were seropositive. All of these seropositivity rates are relatively high.

When Dr. Guillermo Madico arrived in spring of this year he brought with him additional samples from Peru which included 52 urine samples and 142 capillary blood samples

2. Development of detection tests

We have developed a PCR (polymerase chain reaction for DNA amplification) technique capable of detecting *C. trachomatis* and *C. pneumoniae* genomes in clinical samples. Suitable primers have been designed and synthesized, and conditions have been established which allow for detection of the equivalent of 1 infectious unit. These experiments were greatly accelerated by the work of Dr. Guillermo Madico in our laboratory during his visit in March/April of this year. He continued these experiments upon his return to Lima and trained other laboratory personnel in their use.

C. Scientific Impact of Collaboration

The collaboration with our colleagues in Peru makes possible testing of a greater number of positive samples. (It has been shown in preliminary small scale surveys that there is a high rate of infection with Chlamydia in Peru.) However, more importantly, it ensures that the tests being developed will be as accurate for samples obtained from different populations from an area of the developing world as they are for samples obtained from Israel. Furthermore, there is significant input especially in the development of the PCR for detection of chlamydia, since one Peruvian investigator has received training at one of the most advanced PCR laboratories in the world as well as in our laboratory. It should be noted that very little is known about the prevalence of chlamydial infections in South America, and very few studies, if any, in which distinction was made between infection

with *Chlamydia trachomatis* and infection with *C. pneumoniae*, have been carried out on populations in this part of the world. From this collaborative study it has already become clear that chlamydial infections are very widespread in Peru, and due notice needs to be taken of this fact when treatment modalities of severe respiratory infection and genital tract infection are being considered. These health considerations and their ramifications were discussed by Prof. Gilman and Prof. Friedman during her recent visit to Peru.

D. Description of Project Impact

Today the serologic methods available for diagnosis of chlamydial infection are based largely on *C. trachomatis* L2 antigen, which does not detect antibodies to *C. pneumoniae* in a reliable manner. The only accepted test capable of differentiating between antibodies to the different species of chlamydia is the microimmunofluorescence (MIF) test, which is not suitable for large scale testing and requires great expertise for proper reading of the test results. A serologic assay in the ELISA format capable of distinguishing between antibodies to *C. trachomatis* and antibodies to *C. pneumoniae* will greatly increase our ability to track both kinds of infections and assess their impact on the health of inhabitants of developing areas. The results of this project are not yet being applied on a large scale. However, it is already becoming clear that not all high titers to *C. trachomatis* L2 antigen in persons with suspected STD can be attributed to *C. trachomatis*, but that some may well be due to *C. pneumoniae* infections. Furthermore, it seems that from the age of 2 years, *C. pneumoniae* may be associated with severe respiratory illness in children in Peru, and that infection with *C. trachomatis* is more widespread than in many population groups in Israel. We are now convinced that given appropriate resources, our Peruvian counterparts will be able to contribute significantly to the fight against chlamydial disease in their area.

E. Strengthening of Developing Country Institutions

With funds available from this grant (supplemented by funds from additional grants) the Peruvian laboratory, which is physically located in the Department of Pathology of the Universidad Peruana Cayetano Heredia, has been supplied with a CO₂ incubator, a -70°C. freezer, and laminar flow hoods. These will eventually allow for chlamydial antigen production, although at least initially, the Peruvian scientists will receive Chlamydial antigen from the laboratory in Beer Sheva. Maritza Calderone and Dr. Guillermo Madico have now spent 3 months and 5 weeks, respectively, at Ben Gurion University, in the Virology laboratory. Upon their return to Peru, both began

immediately to apply the techniques developed in Beer Sheva, and to adapt them to their own working conditions as well as to train both students and technicians in their use. In June, 1996, Prof. Friedman travelled to Lima to observe the implementation of the techniques and assist in troubleshooting. While there she presented a departmental seminar on Chlamydia, their growth, and their involvement in various clinical syndromes.

F. Future Work

During the coming final year of this project, we intend to further explore the flexibility of certain parameters of the ELISA assay, complete development of the PCR assay and test all samples collected for chlamydia detection, collate the data, and draw conclusions. The project is basically on schedule, although we had hoped to have completed development of the PCR technique by now. (This was unavoidably delayed by several unrelated factors.) Optimization of the PCR technique will take place both in Peru and in Beer Sheva, with communication by Email and by fax as appropriate.

Our Peruvian colleagues have shown that PCR is definitely within their capabilities, using agarose gel electrophoresis for identifying amplified sequences. However, we believe that the PCR technique can be adapted for simpler detection of probe hybridization to amplified sequences by ELISA techniques, and this is one of our aims for the coming year.

Section II:

A. Managerial Issues

No new managerial issues have arisen since the last report.

B. Budget

There are no new budget issues.

C. Special concerns

In general, protocols addressing special concerns have not changed; we have come to the conclusion that a patent application is not feasible for the ELISA developed or for the PCR. No plans have yet been made for possible commercialization of either of the processes.

D. Collaboration, Travel, Training, and Publications

Maritza Calderone spent close to three months in our laboratory in Beer Sheva. She brought with her serum samples from children with respiratory infections, from control children, and from women with gynecologic problems to be tested with our ELISA, as well as nasal washes, urine samples, and genital swabs to be tested for the presence of chlamydia by PCR. During her stay she learned the ELISA technique as well as preparation of all the reagents and tested the sera which she brought with her from Peru. She brought back to Peru sufficient chlamydial antigen for an extended period of time until we will supply more or they will be able to produce their own.

Dr. Guillermo Madico spent five weeks in our laboratories. He brought with him additional serum and urine samples and while in Beer Sheva refined the PCR technique so that he was able to harvest DNA from very dilute samples. Upon his return to Lima he trained Maritza in the refined technique, which she can now perform routinely.

Prof. Maureen Friedman visited the laboratories in Peru during June of this year. She was impressed with the ability of our Peruvian collaborators to accomplish scientifically sophisticated techniques in much poorer physical conditions than are usually expected in diagnostic laboratories. This is a tribute not only to the ingenuity of the laboratory personnel and students, but also to their intense determination to succeed. Her trip was covered only in part by the grant.

The first scientific article describing the ELISA serologic technique has been submitted for publication, and several more are planned, including some from an epidemiologic perspective.

E. Request for AID or BOSTID actions

At this time we have no special requests for AID or BOSTID action.