SEXUALLY TRANSMITTED DISEASES IN THE HIV ERA

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Sexually Transmitted Diseases in the HIV Era

Epidemiology and Costs of STDs

D5-001 STD TRANSMISSION DYNAMICS AND CONTROL, Robert C. Brunham, University of Manitoba, Manitoba, Canada.

Sexually transmitted infections (STIs) are a distinct cluster of infectious diseases that share a common epidemiologic paradigm. From a transmission point of view, infection is restricted to sexually active individuals and the pathogen is predominantly found in the genital tract of infected persons. The pathogens survive poorly outside the human body and require direct contact for transmission. STIs are an important cause of acute disease morbidity including genital discharges and genital ulcerations. They are also an important cause of adverse pregnancy outcome, poor reproduction and genital cancers. Although many microbial agents can be transmitted sexually, the eight major sexually transmitted pathogens are *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*, *Haemophilus ducreyi*, herpes simplex virus type 2, human papilloma virus, hepatitis B virus, and human immunodeficiency virus. As a group, these pathogens are the target for considerable resources in public health programs for disease control.

The epidemiology of sexually transmitted infections is dominated by the concept of the reproductive rate of infection, $R_o = ßcd$. R_o defines the number of second generation infections produced by a single infected individual in a fully susceptible population. This number needs to exceed 1 for productive infection chains to be sustained, and for infection to spread within a population. R_o, in turn is determined by the transmission efficiency, ß, the duration of infectivity, d and the contact rate between spreaders and susceptibles, c. Because stable biological attributes of the organism principally determine ß and d, variation in STD rates are primarily determined by variations in human sexual behaviour. In order for STIs to maintain productive infection chains, sexual partner change rates need to be considerably in excess of the average rate found in a population and because of this, STDs are clustered into subgroups within the population termed core groups. They serve as the reservoir of infection and are the source for STI to the remainder of the population.

Control of STIs depends on reducing the reproductive rates to < 1 by identifying and focusing on core groups and by targetting one or more of the three determinants of R_0 (ß,c,D) for control effort. Two programs which have been successful in control of STDs will be discussed in detail to demonstrate how manipulation of key parameters that determine the reproductive rate for STIs can result in remarkable control. The examples are drawn from the Thailand STD control program and the Manitoba chlamydial control program. Analysis of these public health programs is used to highlight the notion that STD control is feasible within available resources when programs are properly targeted.

D5-002 CURRENT STD THERAPIES: ADVANTAGES, LIMITATIONS, AND THE FUTURE. H. Hunter Handsfield, University of Washington and Seattle-King County Department of Public Health, Seattle, Washington.

Principles of STD treatment include: making an etiologic diagnosis when practical; use of single-dose therapy if available; empirical therapy for additional, undiagnosed infections; treatment of sex partners; awareness of local patterns of antimicrobial susceptibility; avoidance of the minimum effective dose, especially for gonorrhea or chancroid; cost, convenience, and likelihood of follow-up will influence treatment choice. Treatment of gonorrhea has recently been shown to reduce urethral HIV shedding in HIV-infected patients, supporting the hypothesis that non-ulcerative STDs enhance the risk of HIV transmission. **Gonorrhea:** Antimicrobial resistance in *N. gonorrhoeae* includes plasmid-mediated ß-lactamase production; chromosomal resistance to multiple antibiotics; plasmid-mediated tetracycline resistance; spectinomycin resistance; and high- and low-level quinolone resistance, a growing problem in some areas. Recent investigations of therapy for anogenital gonorrhea have emphasized single-dose oral cephalosporins or fluoroquinolones. In 4 studies combined, cefixime 400 mg cured 97.7% (CI_{95} 96.1-99.3%); 800 mg was no more effective. Cefpodoxime proxetil 200 mg cured 96.5% (CI_{95} 94.3-98.6%). In 9 studies, ciprofloxacin 250 mg cured 99.2% (CI_{95} 98.6-99.8%); 500 mg gave no additional benefit. Ofloxacin 400 mg cured 98.3% (CI_{95} 97.3-99.3%). Temafloxacin, fleroxacin, pefloxacin, and enoxacin gave cure rates of 96-99%. **Chlamydia and NGU:** Apparent tetracycline-tolerant isolates have been described but are not related to treatment outcome; for practical purposes, all the tetracyclines and macrolides are active against *C. trachomatis*, but susceptibility to the quinolones is variable. Recent studies have emphasized single-dose azithromycin or 7-10 d quinolone regimens. Azithromycin 1.0 g cured 96% (CI_{95} 93.4-99.5%) of uncomplicated *C. trachomatis* infections. Ofloxacin for 7-10 d (but not ciprofloxacin) is effective; fleroxacin is effective but intolerance (photosensitivity) is common. Azithromycin 1.0 g is effective against nonchlamydial NGU. **Chancroid:** Increasing resistance limits utility of some traditional therapies, but the macrolides and quinolones remain active. Recent studies have emphasized single-dose therapy with ceftriaxone, single-dose azithromycin, 3-day therapy with the fluoroquinolones, and 7-day courses of erythromycin; all remain effective (80-90%) in most geographic areas, but cure rates for all regimens are impaired in HIV-infected patients. **Syphilis:** Penicillin remains the treatment of choice for all stages of disease, although clinical failures are not rare. **Pelvic inflammatory disease:** Despite controversy about the frequency or importance of anaerobic pathogens, most authorities recommend therapy to cover anaerobes as well as *N. gonorrhoeae* and *C. trachomatis*; ofloxacin plus metronidazole or clindamycin is commonly recommended for outpatient therapy. **Vaginal infections:** Single-dose metronidazole 2.0 g remains the treatment of choice for trichomoniasis. New alternatives for bacterial vaginosis are vaginal metronidazole and vaginal or oral clindamycin. Single-dose oral fluconazole is a new alternative to multiple-dose therapy with imidazole creams for vulvovaginal candidiasis. **Genital herpes:** Famciclovir (a prodrug of penciclovir, the parenteral preparation) and valacyclovir (an acyclovir prodrug) are new alternatives to acyclovir; their primary advantages are in dosage convenience. The manufacturer has proposed over-the-counter sales of acyclovir, a controversial recommendation that seemingly would do more harm than good. **Genital warts:** Local ablative therapy remains the general approach; no treatment eradicates HPV or is known to modify the risk of later neoplasia.

Chlamydial Screening Strategies

D5-003 DETECTION OF *CHLAMYDIA TROCHOMATIS* AND *NEISSERIA GONORRHOEAE* UROGENITAL INFECTIONS IN URINE BY LIGASE CHAIN REACTION: A NEW APPROACH TO STD DIAGNOSIS, Helen H. Lee, Shanfun Ching, Yoshihiro Ohhashi, Yanfeng Yang, Patricia Plier and Omar Khalil, Probe Diagnostics Business Unit, Diagnostics Division, Abbott Laboratories, Abbott Park, IL 60064

Sexually transmitted diseases (STD) have been ranked as the second most important disease worldwide for which intervention is possible among women 15-44 years of age. Accurate screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) has become increasingly important in public health effort to control STD. Ligase Chain Reaction (LCR) is a new molecular non-culture method which amplifies specific nucleic acid sequences. Four single-stranded probes are designed such that probes 1 and 3 are complementary to the 3' and 5' halves respectively of the plus target strand, while probes 2 and 4 to the minus strand. Amplification is achieved by repeated cycles of heating and cooling for strand separation and specific hybridization. Amplicons are detected via automated immuno-capture by labeling the ends of each probe pair with different capture and detection haptens. Carry-over contamination is prevented by chemical inactivation post detection. In addition, amplicon detection is carried out in a closed automated instrument system in which reactions are manipulated by piercing the amplification tube.

Both GC and CT LCR assays use the same sample preparation procedure which consists of a simple heating step for 15 min. at 97°C. The GC LCR assay detects an OPA-2 gene sequence, whereas the CT LCR detects a sequence of the plasmid gene. The specificity of the probe sets for both organisms has been extensively validated. GC LCR assay detected 136/136 *N. gonorrhoeae* isolates, but was negative for 58 non-*Neisseria* microorganisms and 84 non-GC *Neisseria* strains. The CT LCR detected all 15 *C. trachomatis* serovars, but not 57 non-CT microorganisms. Extensive clinical trials were carried out at 8 sites in 1,043 male and 943 female urine for CT; 831 male and 575 female urine for GC. Inhibitory substance(s) were present in ~ 1-5% of specimens. Despite sample inhibition, the sensitivity of LCR in male and female urine is 93.5% and 95.7% respectively for CT ; 99.1% and 95.1% respectively for GC. LCR specificity ranged from 99.8 to 100%. Compared to culture of endocervical swabs in the same patient, LCR in urine detected 36.1-37.0% (CT) and 1.0-4.5% (GC) more true positives. The increased sensitivity, reduced assay time and automation make LCR a highly effective routine clinical laboratory test for the detection of CT or GC genirtourinary tract infections in symptomatic or asymptomatic individuals using a non-invasive sample.

D5-004 NONCULTURE TESTS FOR THE DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTIONS, Walter E. Stamm, University of Washington School of Medicine, Seattle, WA

The last decade has witnessed the development of successful nonculture methods for the diagnosis of C. trachomatis infection, including direct antigen detection by immunofluorescence (DFA) or enzyme immunoassay (EIA), use of nucleic acid probes, and more recently, use of the DNA amplification methods polymerase chain reaction (PCR) and ligase chain reaction (LCR). Of the antigen detection tests, the DFA, when optimally performed utilizing monoclonal antibodies to the chlamydial MOMP, demonstrates the best sensitivity and specificity among antigen detection tests. The test is operator dependent, however, and well suited to testing only small numbers of specimens. Most of the EIAs available are now highly specific due to the recent introduction of confirmatory assays. EIA sensitivity, however, varies with the individual test and ranges from 60-80% for cervical and urethral specimens. The EIA assays are generally not sensitive enough to use when testing urines in men and are both insensitive and nonspecific when testing urines from women. Non-amplified DNA hybridization assays have demonstrated sensitivities and specificities similar to the DFA and some EIAs.

PCR and LCR provide a major increment in sensitivity as compared with previous nonculture tests. Since the sensitivity of LCR/PCR generally exceeds that of culture, most recent studies now utilize a new standard of comparison for determining sensitivity, namely a composite gold standard in which either a culture-positive specimen or a culture-negative, probe positive specimen that can be confirmed by DFA cytospin and/or MOMP probe constitutes a true positive. Utilizing the PCR and LCR, it has become clear that the sensitivity of culture for chlamydia varies very widely and may often be only in the 50-60% range. Optimally, culture provides an 80-90% sensitivity compared with the composite gold standard. Studies utilizing PCR and LCR demonstrate that both assays achieve excellent sensitivity (generally >90%) and specificity (generally >99%) using male urethral or male urine specimens. Similar results have been reported using LCR with female endocervical speicmens, while the sensitivity of PCR has sometimes been lower with female cervical specimens due to presumed inhibitors. Surprisingly, PCR and LCR also appear to provide sensitivities of 90-95% and high specificities utilizing female urine specimens. The obvious advantages resulting from urine collection as compared with collection of urethral and cervical swabs should allow greatly expanded testing for chlamydia in a wider variety of clinical settings using LCR or PCR.

Finally, LCR/PCR have other potentially important uses, including testing of specimens from the upper genital tract (where sensitivity of culture and other assays is poor), studies of transmission, evaluation of persistent infection, and use in field studies. Additional studies are needed to define optimal strategies for urine screening, to determine the cost-effectiveness of these strategies, to develop rapid tests, to overcome the problem of inhibitors, to assess sensitivity and specificity in low prevalence and non-research settings, and to define the actual clinical and epidemiological meaning of positive tests in various circumstances.

STD Diagnostics: Viral and Other Bacterial Infections

D5-005 HERPES SIMPLEX VIRUS DIAGNOSIS, Rhoda L. Ashley, University of Washington School of Medicine, Seattle.

Despite increased awareness of HSV-1 and HSV-2 as sexually transmitted pathogens and the increase in HSV-2 seroprevalence in the past decade, genital herpes infections are underdiagnosed. In over half of transmission events to sexual partners and in over 70% of neonatal herpes cases, the source contact is unaware of being infected. Over 20% of the general population of the U.S. and as many as 60-80% of HIV infected individuals are seropositive for HSV-2, yet most deny a history of genital herpes. Several recent studies have indicated that this may be a function of lack of recognition of signs and symptoms of genital herpes by both patients and practitioners. For optimal and cost effective management and for directed counseling efforts to prevent the continued spread of genital HSV, clinicians must determine when and how to seek a diagnosis of genital herpes in the asymptomatic patient and must be able to recognize unusual as well as classical clinical manifestations of genital HSV infection.

The virology laboratory can play a key role in these efforts. The gold standard diagnostic test is viral isolation in cell culture with subsequent identification and typing. Modified formats combine viral amplification and definitive identification steps to decrease the time to diagnosis without loss of sensitivity. The most rapid detection tests involve detection of HSV antigen or DNA directly from the specimen, without viral amplification. These tests require only hours to perform but their sensitivity is directly related to the amount of virus in the sample. While direct antigen or DNA detection tests can be as sensitive as viral culture when lesions are sampled, detection of low titers and of asymptomatic HSV shedding by these methods is difficult. HSV-PCR, which exponentially amplifies selected viral DNA segments in the sample before a DNA detection step, is highly sensitive and is, therefore, the test of choice for diagnosing HSV in cerebrospinal fluid from patients with encephalitis. HSV-PCR can detect low titers of HSV in healing lesions and in asymptomatic patients. Preliminary indications from studies performed by our group are that HSV DNA presence correlates with the presence of viable HSV in patient specimens. However, the association between infectivity and presence of HSV DNA by PCR remains to be determined.

Detection of antibodies can be of value in documenting seroconversion in seronegative patients. Type specific serologies based on Western blot or on the type specific proteins, gG or gC-1, can document seroconversion to HSV-2 in the HSV-1 seropositive patient. However, serologic methods used by most commercial laboratories cannot accurately distinguish antibodies to HSV-1 from those to HSV-2. Until commercial suppliers incorporate recent advances in the knowledge of HSV antibody responses into their test formats, accurate serologies for identifying silent HSV-1 and HSV-2 infections will be limited in availability.

D5-006 DIAGNOSIS OF HUMAN PAPILLOMAVIRUS INFECTION BY DETECTION OF VIRAL DNA AND BY SEROLOGY

Denise A. Galloway[1,2,3], Joseph J. Carter[1], Greg Wipf[1], Michael E. Hagensee[1,4], Long-fu Xi[1,5], Shu-Kuang Lee[2,5], Jane Kuypers[3], D. Heather Watts[6], King K. Holmes[2,4], Laura A. Koutsky[2,5] and Nancy Kiviat[3]. [1]Fred Hutchinson Cancer Research Center, [2]Center for AIDS and STDs and Departments of [3]Pathology, [4]Medicine, [5]Epidemiology and [6]Obstetrics and Gynecology, University of Washington, Seattle, WA.

Human papillomaviruses (HPVs) are ubiquitous pathogens that commonly infect the genital tract, even in populations that are at low risk for other sexually transmitted diseases. Expression of viral genes results in the stimulation of cellular DNA replication, and disruption of the cell cycle checkpoints that maintain the integrity of the cellular genome. These HPV - induced changes in the cell are the basis of the cytological and histological changes that are characteristic of HPV infections. There are no in vitro culture systems that allow propagation of HPVs, and HPVs have not been classified based on their serological relatedness. Instead HPVs are grouped into types, subtypes and variants based on DNA sequence homology. The definitive diagnosis of HPV infection is based on the detection of viral DNA in clinical samples. Many detection schemes are in use; perhaps the most widely used in research settings is PCR amplification of a conserved segment of the L1 gene followed by hybridization with a generic probe that has homology with most (> 30) genital HPV types, and hybridization with probes to specific HPV types. Using this approach we have examined the prevalence of HPV in several different cohorts: for example, among college women with few sexual partners and low rates of other STDs, the prevalence of genital HPV infection at the initial visit was 31%; among pregnant women of low socioeconomic status 57% had detectable HPV DNA; and among a population of homosexual men HPV DNA was detected in 87% of anal specimens. Analysis of PCR products amplified from the non-coding region of the HPV genome, by sequencing or SSCP, now permits the detection and characterization of individual variants. Recent studies have demonstrated the potential to diagnose HPV infections by serological assays. Antibodies react "type-specifically" with a conformational epitope(s) on the L1 protein of the viral capsid. We have developed a capture ELISA using monoclonal antibodies to an external epitope on the L1 protein and HPV capsids produced from recombinant vaccinia viruses. Our studies have shown that HPV seropositivity is strongly associated with current or historical HPV infection, with detection of HPV DNA in the genital tract, and with number of sexual partners. Longitudinal studies have found that seroconversion is preceded by the initial detection of the homologous type of HPV DNA, new partners and a transient IgM response.

D5-007 NEW DIAGNOSTIC APPROACHES TO GENITAL ULCER DISEASES, Stephen A. Morse, Centers for Disease Control and Prevention, Atlanta, GA 30333.

Genital ulceration is defined as a genital lesion characterized by a defect in the epithelium of the skin or mucosa. Sexually transmitted infections characterized by genital ulcerations include syphilis, chancroid, herpes, lymphogranuloma venereum and donovanosis. However, the clinical presentation of these infections is diverse, multiple infections are common and their laboratory diagnosis is technically demanding. The importance of these infections has increased tremendously with evidence that genital ulceration is a major risk factor for the transmission of HIV. Genital ulcerative diseases are relatively more frequent in developing countries than in industrial countries. In addition, the etiology of genital ulcer disease varies geographically. Genital herpes is the most common cause of genital ulcers in North America and Europe, chancroid is prevalent in parts of Africa and Latin America, and donovanosis occurs frequently in Papua New Guinea, India, the Caribbean and Africa. Other than serological tests for syphilis, laboratory tests for the diagnosis of genital ulcerative diseases are not widely available. In addition, many existing tests are insensitive or are difficult to perform. In many instances, the diagnosis of a genital ulcerative disease is based predominantly on the clinical presentation of the ulcer itself. This results in misdiagnosis of genital lesions; insensitivity of current diagnostic tests has resulted in the inability to identify a specific etiologic agent. It has become increasingly apparent that the most common agents responsible for genital ulcerative diseases in North America (Treponema pallidum, herpes simplex virus types-1 and -2, and Haemophilus ducreyi) are sometimes clinically indistinguishable. Recently, there has been an increased demand for nonculture methods for the detection and identification of the etiologic agents responsible for genital ulcers. PCR offers a new approach to the diagnosis of genital ulcers. PCR is more rapid, sensitive and specific than currently used tests. Multiplex PCR assays can simultaneously amplify DNA targets from multiple agents, which may be present in a single lesion. The results of PCR assays for T. pallidum, H. ducreyi and herpes simplex virus types-1 and -2 will be important in establishing geographic differences in the causes of genital ulcers and in the development of control programs.

STD Pathogenesis: Genital Ulcer Diseases

D5-008 CELLULAR IMMUNE RESPONSE TO TREPONEMA PALLIDUM INFECTION, Sheila A. Lukehart, Lynn Barrett, Thomas Arroll, Jeanne Shaffer, James Nasio, Francis Plummer, and Wesley Van Voorhis, University of Washington, Seattle WA 98195 and University of Nairobi, Nairobi, Kenya.

The manifestations of early syphilis infection resolve spontaneously, while asymptomatic systemic infection persists. The immune mechanisms responsible for local bacterial clearance have been under investigation in our laboratory, and we have hypothesized that antigen-sensitized CD4+ cells release macrophage activating cytokines, such as interferon-γ, that attract and activate macrophages. In the presence of opsonic antibody, these macrophages then phagocytize and kill T. pallidum. To test this hypothesis, biopsies from primary and secondary syphilis lesions have been examined by immunohistochemistry for infiltrating cells, and by reverse transcriptase polymerase chain reaction (RT-PCR) for cytokine mRNA; T. pallidum-reactive T cell lines have been derived directly from primary chancres. Immunohistochemical staining of lesions reveals that T lymphocytes predominate, including both CD4+ and CD8+ cells. Macrophages are abundant, and only rare NK cells are seen. RT-PCR analysis reveals a consistent cytokine profile: IL-2 and IFN-γ mRNA are found in both primary and secondary lesions, while IL-4 is absent; IL-10 mRNA is seen in variable amounts. This profile is consistent with a Th1-type CD4+ response, although the cellular source of each cytokine has not yet been determined. IL-10 can be produced by Th2 cells, however the lack of IL-4 argues against a Th2 response, and IL-10 may be produced by the infiltrating macrophages. T lymphocytes expanded by culture from primary lesions contain both CD4+ (51%) and CD8+ (44%) lymphocytes, consistent with immunohistochemical staining, and a high proportion of derived T cell lines are T. pallidum-reactive. Two antigen-specific T cell lines investigated to date are MHC-restricted, CD4+, and possess αβ-T cell receptors. By RT-PCR, these lines have a mixed cytokine profile, consistent with either Th0 phenotype or a mixed cell line. In vitro studies demonstrate that T. pallidum are opsonized by antibodies directed against epitopes found only in pathogenic treponemes, and are killed by macrophages following phagocytosis. The few organisms that persist at the site of the local lesions following bacterial clearance are resistant to phagocytosis by macrophages, even in the presence of antibody.

D5-009 CHARACTERIZATION OF *TREPONEMA PALLIDUM* OUTER MEMBRANES AND RARE OUTER MEMBRANE PROTEINS, Justin D. Radolf[1,2], Esther Robinson[1], Kenneth Bourell[1], Minyue Li[1], Darrin R. Akins[2], Taissia G. Popova[2], Stephen F. Porcella[2], Jeffrey D. Jones[2], David L. Cox[3], and Michael V. Norgard[2]. Departments of [1]Internal Medicine and [2]Microbiology, U.T. Southwestern Medical Center, Dallas, TX 75235 and [3]Centers for Disease Control and Prevention, Atlanta GA 30333.

Treponema pallidum, the agent of venereal syphilis, has a remarkable ability to evade host defenses. We have proposed that the molecular architecture of the *T. pallidum* outer membrane plays a major role in the bacterium's immunoevasiveness. Freeze-fracture electron microscopy, a major technique in these studies, has revealed that the *T. pallidum* outer membrane contains only rare transmembrane proteins whose distinctive distribution suggests that their lateral mobility within the lipid bilayer is restricted. These rare outer membrane proteins are believed to be prime candidates for a syphilis vaccine; their contribution(s) to treponemal virulence and physiology also need to be determined. Other evidence also suggests that these proteins react poorly or not at all with syphilitic sera. In contrast, the major *T. pallidum* immunogens, molecules formerly thought to be surface-exposed, are periplasmic proteins predominantly anchored by N-terminal lipids to the periplasmic leaflet of the cytoplasmic membrane. Two approaches currently are being used to characterize these rare outer membrane proteins. The first involves fractionation of virulent *T. pallidum* and isolation of outer membranes as a source of proteins for generating DNA and antibody probes for molecular cloning and characterization. The second involves the expression of *T. pallidum* export signals as fusions with an alkaline phosphatase reporter gene. Also important for these efforts has been the development of a highly sensitive technique for localization of surface-exposed treponemal proteins which involves encapsulation of *T. pallidum* in porous agarose beads (gel microdroplets). Lastly, lipid analysis of isolated *T. pallidum* outer membranes has revealed an absence of antigenic lipids, most notably cardiolipin. These data indicate that *T. pallidum* has evolved an ultrastructure in which both protein and lipid constituents are inaccessible to host antibodies.

D5-010 *HAEMOPHILUS DUCREYI* PATHOGENESIS AND HUMAN EXPERIMENTATION, Stanley M. Spinola[1], Antoinette F. Hood[1], Attilio Orazi[1], Janet N. Arno[1], Patricia Kotylo[1], Michael A. Apicella[2], Anthony A. Gaspari[3], Linda M. Wild[4] and Anthony A. Campagnari[4]. [1]Indiana University, Indianapolis, IN, 46202, [2]University of Iowa, Iowa City, [3]University of Rochester, Rochester, and [4]SUNY at Buffalo, Buffalo.

Haemophilus ducreyi is the etiologic agent of chancroid. Due to a lack of longitudinally collected specimens, almost no information exists on the pathogenesis of and human immune response to *H. ducreyi*. To study host-bacterial interactions, we developed a human challenge model of *H. ducreyi* infection. Live and heat-killed suspensions of *H. ducreyi* were injected into the skin of the upper arm with a Multi-Test Applicator (Lincoln Diagnostics, Decatur, Illinois). Studies done by the manufacturer suggest that the applicator reproducibly injects approximately 1/1000 of solutions loaded on its pins to a depth of 1.9 mm in the skin. In dose response studies, 10 subjects were challenged at a total of 30 sites, and none developed fever, lymphadenopathy or disseminated infection. To minimize risks to the subjects, infection was terminated by administration of antibiotics when lesions became pustular. In initial studies, pustules developed within 24 hours at sites where 10^5 to 10^6 CFU were placed on the device and inoculated into the skin. Although only 10^2 to 10^3 CFU were probably introduced into the skin, the experimental lesions progressed too rapidly to mimic naturally occurring disease, and the subjects were treated at 3 days (1). We have now refined the model so that the course of infection mimics natural disease and subjects are infected for 14 days. Application of suspensions containing as few as 1.5 x 10^3 CFU caused erythematous papules at 3/6 sites; 1 of these lesions progressed to pustule while 2 lesions resolved. Suspensions containing 7.5 x10^3 CFU to 1.5 x 10^4 CFU caused erythema at 6/6 sites; 2 of these lesions progressed to pustule while 4 resolved. Thus, injection of as few as 1-2 CFU may cause disease, and 7 to 15 CFU reliably cause infection. On day 14, pustular lesions were biopsied, and contained 4 to 9 x 10^3 CFU of *H. ducreyi* /gm of tissue. At 14 days, there was an infiltrate of Langerhans cells in the skin, and the epidermis contained pustules. In the dermis, there was a predominant perivascular and an interstitial infiltrate of CD4+ T-cells and macrophages, and capillaries were lined by reactive endothelial cells. The T-cells and keratinocytes expressed HLA-DR, which was consistent with a delayed type hypersensitivity response. Although infection elicited a cutaneous cellular immune response, there were no changes in circulating lymphocyte subsets and no evidence that infection elicited PBMC responses to *H. ducreyi* antigens. The volunteers failed to mount humoral responses to bacterial cells, OMPs and LOS. We conclude that experimental infection with *H. ducreyi* is well tolerated and safe. The focus of the host response at 3 and 14 days is cutaneous cellular infiltrate, which is sometimes effective in clearing infection. Recruitment of activated CD4+ T-cells and macrophages into the skin may partially explain the association between chancroid and HIV transmission.

1. Spinola, S.M., Wild, L.M., Apicella, M.A., Gaspari, A.A., and Campagnari, A.A. 1994. Experimental human infection with *Haemophilus ducreyi*. J. Infect. Dis., **169**:1146-1150.

STD Pathogenesis and Mucosal Inflammation

D5-011 LIPOOLIGOSACCHARIDE SIALYLATION AND VIRULENCE OF *NEISSERIA GONORRHOEAE*, Michael A. Apicella[1], Robert Mandrell[2], Peter A. Rice[3], Frank N. K. Lee[1], Daoguo Zhou[1] and Milan Blake[4], [1]University of Iowa College of Medicine, Iowa City, IA 52242, [2]Oakland Children's Hospital, Oakland, CA, [3]Boston University School of Medicine, Boston, MA, [4]Rockefeller University, New York, NY.

Neisseria gonorrhoeae is a strict human pathogen which has adapted remarkably well to survive in the human host. One of the most interesting strategies used by the organism to evade the host immune response is the expression of lipooligosaccharide (LOS) structures which are immunochemically identical to human glycosphingolipids, including sialoparagloboside, paragloboside, asialo-G3, LacCer and P*K*. It has been shown that the organism can sialylate the paragloboside moiety in vivo and in the presence of exogenous CMP-NANA during in vitro growth. The gonococcus expresses a sialylotransferase which can be found in periplasmic and outer membrane preparations but lacks enzymes capable of synthesizing CMP-NANA. Therefore, it must parasitize host sources for this substrate during infection. Biologic studies indicate that gonococci freshly isolated from infected human exudates or grown in the presence of CMP-NANA are resistant to killing by normal human serum. In addition, it has been demonstrated that organisms grown in the presence of CMP-NANA are no longer killed by anti-porin and opacity associated protein (opa) monoclonal antibodies capable of killing the non-sialylated strain. Sialylation of the LOS interferes with LOS-opa binding. Desialylation with neuraminidase returns binding to pre-sialylation levels. Invasion and attachment studies in model systems of *N. gonorrhoeae* grown in the presence and absence of CMP-NANA indicate that gonococci with sialylated LOS adhere and invade significantly less well than gonococci with non-sialylated LOS. Recent studies in human experimental gonococcal infection indicate that gonococci grown in the presence of CMP-NANA are significantly less infectious than organisms grown in the absence of this substrate. Confocal and immunoelectron microscopic analysis of infected urethral exudates from males with gonorrhea demonstrate that approximately 90% of the LOS is sialylated. Gonococci can be found within urethral stratified squamous epithelial cells and PMN's. Acridine orange staining reveals that the majority of organisms within the urethral stratified squamous cells are viable while relatively few within the PMN's are alive. These studies indicate that LOS sialylation may be an important virulence factor in protecting the organism against host immune defenses. It does not appear to play a significant role in adherence and invasion of host genital tract cells.

D5-012 PATHOGENESIS OF EXPERIMENTAL HUMAN INFECTION WITH *NEISSERIA GONORRHOEAE*, Janne G. Cannon[1], Myron S. Cohen[1], Susan F. Isbey[1], Ann E. Jerse[1], Leesa G. Whicker[1], Jo Ann F. Dempsey[1], Andrea B. Wallace[1], David Johnston[1], Terri L. Snodgrass[1], and H. Steven Seifert[2], [1]University of North Carolina, Chapel Hill, NC, and [2]Northwestern University Medical School, Chicago, IL.

One reason it has been difficult to address fundamental questions concerning the pathogenesis of gonorrhea is the lack of appropriate animal models for gonococcal infection. Gonococci are highly adapted for human hosts, which raises concerns about the relevance of conclusions drawn from studies using other host species. To circumvent this limitation, a number of studies have used experimental infection of male volunteer subjects. Experimental gonorrhea is similar in clinical features and time course to naturally acquired disease; over five hundred subjects have been infected in several separate studies without significant reported complications. The goals of these studies have involved description of phenotypic variation of the organisms *in vivo*, testing of vaccine candidates, and examination of virulence factors of the organisms. In our studies, we have inoculated over 80 subjects, nearly half of whom became infected. The ID80 for strain FA1090 is approximately 10^6 colony-forming units. We characterized antigenic variation of surface components of strain FA1090, including Opa outer membrane proteins and pilin, during experimental infection. The objective of these experiments was to determine if expression of particular antigenic variants is favored during uncomplicated infection. We observed extensive variation in expression of both Opa proteins and pilin *in vivo*, although no single "preferred" variant of either surface component could be identified. We have begun to examine gonococcal virulence factors through the use of isogenic mutants of strain FA1090. To determine if the extensive variation in pilin expression that occurs in early infection is essential for infectivity, we constructed a *recA* mutant of FA1090 that was unable to vary pilin expression, since such variation requires RecA-mediated recombination. The *recA* mutant caused infection, although fewer subjects infected with FA1090 *recA* developed a urethral discharge, relative to those infected with the wild type strain. We conclude that pilin variation is not required for gonococcal infection in male subjects. Construction of mutants unable to express putative gonococcal virulence factors is complicated by the necessity to avoid introduction of novel antibiotic resistance markers into strains for human challenge. We have therefore developed a genetic strategy for constructing such mutants, using a selection/counterselection gene cassette in a two-step transformation procedure that results in a final strain with the gene of interest inactivated, but no new selectable markers introduced. We are currently constructing mutants that will allow an assessment of the role of several different gonococcal components in virulence in the human challenge model.

D5-013 *CHLAMYDIA TRACHOMATIS* GENITAL TRACT INFECTION IN GENE KNOCKOUT MICE. Richard P. Morrison[1,3], Karen Feilzer[1], and Daniel B. Tumas[2]. [1]Laboratory of Intracellular Parasites and [2]Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT; and [3]Departments of Medicine and Microbiology, University of Alabama at Birmingham School of Medicine, Birmingham, AL.

Gene targeting has been used to produce mice with disrupted β_2 microglobulin ($\beta_2M^{-/-}$), I-A (class $II^{-/-}$) or CD4 ($CD4^{-/-}$) genes. These mice lack cell surface expression of MHC class I, MHC class II or CD4 molecules, respectively. $\beta_2M^{-/-}$ mice are deficient in $CD8^+$ cytotoxic T lymphocytes, class $II^{-/-}$ mice lack helper T cells ($CD4^+$), and $CD4^{-/-}$ mice have reduced levels of helper T cell activity. In this study we examined the capacity of those immunodeficient mouse strains to resolve *Chlamydia trachomatis* genital tract infection. C57BL/6 and $\beta_2M^{-/-}$ mice resolved infection similarly, and were culture negative by 4 to 5 weeks following infection. However, class $II^{-/-}$ mice failed to resolved infection and $CD4^{-/-}$ mice showed a significant delay (2 weeks) in the resolution of chlamydial genital tract infection. Secondary challenge of C57BL/6, $\beta_2M^{-/-}$ and $CD4^{-/-}$ mice established that acquired protective immunity developed, characterized by an infection of shortened duration and reduced shedding of infectious chlamydiae. Serological analysis of C57BL/6 and $\beta_2M^{-/-}$ mice by ELISA revealed no striking differences in anti-EB IgM, IgGl, IgG2a, IgG2b, IgG3 or IgA antibodies, although some differences were observed in the magnitude of the IgG2a and IgG2b responses. Class $II^{-/-}$ mice produced lower titered anti-chlamydial antibodies of all isotypes. The serum antibody responses of $CD4^{-/-}$ mice were similar to C57BL/6 mice except the anti-chlamydial IgA response in $CD4^{-/-}$ animals was delayed by approximately 3 weeks. Analysis of vaginal washes for anti-chlamydial antibodies revealed the presence of IgG2a, IgG2b and IgA in C57BL/6 and $\beta_2M^{-/-}$ mice, no anti-chlamydial antibodies in class $II^{-/-}$ mice, and primarily IgA in $CD4^{-/-}$ mice. Furthermore, the appearance of chlamydial-specific IgA in the vaginal wash of $CD4^{-/-}$ mice was delayed, but its presence coincided with decreased chlamydial shedding and resolution of infection. C57BL/6, class $II^{-/-}$, and $CD4^{-/-}$ mice were tested for chlamydial-specific delayed-type hypersensitivity (DTH) responses. Intense DTH responses developed in C57BL/6 and $CD4^{-/-}$ mice when challenge with antigen, but class $II^{-/-}$ mice failed to elicit positive responses. Those results demonstrated that MHC class II restricted T cell responses, but not class I restricted $CD8^+$ T cell responses, were necessary for the development of protective immunity to chlamydial genital tract infection. Furthermore, a strong correlation was found between the presence of chlamydial specific IgA in vaginal secretions and protective immunity.

D5-014 MOLECULAR MECHANISM OF *CHLAMYDIA TRACHOMATIS* ENTRY INTO EUKARYOTIC CELLS, Richard S. Stephens, Program in Infectious Diseases, University of California, Berkeley, CA 94720 and the Francis I. Proctor Foundation, University of California, San Francisco, CA. 94143

Characterization of the molecules that functionally interact between chlamydiae and their obligate eukaryotic host cells is essential for understanding pathogenesis of *C. trachomatis* infection. Those molecules that interact in attachment of infectious elementary bodies (EB) and mediate entry or invasion of EBs into host cells represent the initial functional interaction. It has been proposed that chlamydiae use a novel trimolecular mechanism of microbial attachment to mammalian host cells in which a chlamydia-synthesized molecular mimic of heparan sulfate is bound to infectious organisms and this complex is bound by a heparan sulfate receptor on host cells (1). For the lymphogranuloma venereum biovariant (LGV), attachment to and consequently infectivity of, host cells is mediated by a heparan sulfate-like molecule bound to the surface of the organism. Evidence for this interaction is 1) attachment is inhibited by heparan sulfate and heparin but not by other glycosaminoglycans (GAGs), 2) receptor analogs for heparan sulfate bind chlamydiae and block attachment, and 3) heparan sulfate lyase (heparitinase) treatment of chlamydiae specifically hydrolyzes a ligand essential for attachment and infectivity. Direct evidence for the role of GAG in attachment of chlamydiae to host cells is that attachment can be restored for heparitinase-treated organisms by coating organisms with exogenous heparan sulfate. Moreover, the infectivity of these organisms is rescued. Similar data have been obtained for the other biovariants of *C. trachomatis*, although the trachoma biovar exhibits additional heparin-insensitive attachment (2). Using mutant eukaryotic cell lines that cannot synthesize GAGs a unique sulfated GAG species is synthesized only in chlamydia infected host cells. This is structurally and functionally related to the chlamydial adhesin ligand in that it is a sulfated GAG, it is sensitive to heparitinase digestion, and it can rescue infectivity of heparitinase-treated organisms. To determine if the chlamydial ligand is only involved in attachment or whether it is also important in subsequent entry events, the chlamydia-eukaryotic cell interaction was modeled using latex microspheres coated with heparan sulfate or native chlamydial ligand. Both compounds coated on beads mediate attachment to cells that is competitively inhibited by chlamydia organisms; likewise, heparan sulfate coated beads competitively inhibit chlamydial infectivity. Significantly, coated beads were endocytosed as determined by a protease resistant bead fraction associated with viable cells that was time and temperature of incubation dependent. Cell endocytosis of coated beads was confirmed by visualization of internalized beads using electron microscopy. It is concluded from these studies that *C. trachomatis* synthesize a molecular mimic of heparan sulfate and exploit a natural eukaryotic pathway to mediate chlamydial entry into host cells.

1. Zhang, J.P. and Stephens, R.S. 1992. Cell 69:861-869.
2. Chen, J.C.R. and Stephens, R.S. 1994. Mol. Micriobiol. 11:501-507.

Microbiological Synergery

D5-015 HSV-2 IN THE GENITAL TRACT OF HUMANS: MORE PERSISTENT THAN INTERMITTENT. Lawrence Corey, M.D., University of Washington, Seattle, Washington, U.S.A.

Despite increased public awareness of STDs the worldwide prevalence of genital herpes simplex virus infections continue to increase. Within the last decade the seroprevalence of genital herpes in the general United States population has risen 31%; with 21.6% of persons over 15 now HSV-2 seropositive. Continued transmission appears related to an underestimation of the frequency of clinical and subclinical reactivation of HSV-2. Among persons who present with clinically symptomatic HSV-2 disease, 98% recurr: males reactivate 20-30% more frequently than females. More than 3/4 of HSV-2 seropositive persons when appropriately counselled have symptomatic, not asymptomatic infection. Using PCR to detect HSV-2 specific DNA in genital secretions, we find HSV-2 DNA only in persons who are seropositive for HSV-2. Antiviral chemotherapy is able to abrogate the frequency and titer of HSV DNA in genital secretions, indicating that the detection of HSV DNA by PCR in genital secretion is a reflection of recent viral reactivation and replication. In a series of studies in which patients have been sampled daily, HSV DNA was detected in genital secretions on 30% of days in which no genital lesions were present versus 5% of days by culture. HSV can reactivate subclinically in vulvar, cervical, perirectal sites independently. Taken together these data indicate that HSV reactivation from sacral nerve root ganglia is very frequent and in some persons chronic. Epidemiologically these data indicate HSV should be approached like a persistent infection rather than thought of as a virus that "infrequently and intermittently" reactivates on mucosal surfaces. While subclinical shedding of HSV occurs, most persons who are HSV-2 seropositive also have symptomatic recurrences. As such, asymptomatic HSV appears to be more a subjective perception than a biological property of HSV-2. Such redirecting of ones thoughts is important in understanding the immunobiology of HSV persistence as well as developing public health and therapeutic strategies for the control of genital herpes.

Prevention of STDs: Specific Methods

D5-016 BARRIER METHODS: THE PREVENTION OF STDS, Nancy J. Alexander, Contraceptive Development Branch, Center for Population Research, National Institute of Child Health and Human Development, NIH, Bethesda, MD.

Male and female condoms are the only barriers that would be expected to prevent the transmission of most STDs. Male condoms are made of latex but the female condom and newer models of the male condom are being made of polyurethane or other synthetic materials that break less easily and have a longer shelf life. Other physical barriers include the diaphragm, cervical cap, and vaginal sponge. Spermicides are chemical barriers to fertilization and STD transmission, and they are often used in conjunction with a physical barrier. Manufacturers have been producing condoms with and without spermicidal lubricants but whether the presence of spermicide improves either contraceptive efficacy or STD prevention is not clear. Testing of barrier contraceptives involves studies of monogamous couples. When the barrier is used correctly for every act of intercourse (perfect use), the pregnancy rates are low. Studies of inconsistent or incorrect use (actual use) reveal higher pregnancy rates. Reported failure rates are as follows: condom, 2.1% to 13.8%; diaphragm, 2.1% to 18.6%; cervical cap, 8.0% to 27.0%; sponge, 17.0% to 24.5%; and spermicide, 0.3% to 36.8%. Although in vitro data strongly suggest that barriers reduce the spread of STDs, knowledge of the efficacy of barriers in preventing STDs comes mainly from epidemiology studies. Condoms have been reported to protect men against infection with two types of bacteria: gonorrhea and *Ureaplasma urealyticum*. Because these bacteria may infect genital epithelium at sites different from genital infection for HIV, protection against HIV cannot necessarily be inferred from studies reporting the risk of acquiring a specific pathogen. Studies in women are even less conclusive. Some studies have revealed no protection against gonorrhea, human papilloma virus infection, nor cervical infection with Chlamydia. The major active ingredient in spermicides is a detergent, nonoxynol-9, and frequent use can cause genital tract lesions; the occurrence of these lesions is dose dependent. The relationship between lesions and STD transmission is incompletely understood. Studies of SIV transmission in monkeys may provide some useful information. Whether barriers are used to prevent pregnancy or the spread of STDs, they are coitally dependent. They must be applied at the beginning of each act of intercourse, and this requires motivation. The development of more acceptable products can increase the chances that they will be used but even if products were available that were 100% effective, a 100% reduction in disease spread would never be achieved. New chemical barriers must be tested in the presence of physical barriers; thus, their absolute efficacy will be difficult to ascertain.

D5-017 MASS TREATMENT STRATEGIES FOR CONTROLLING SEXUALLY TRANSMITTED DISEASES, David H. Martin and Deborah A. Cohen, Louisiana State University Medical Center, New Orleans LA.

While STD rates have dropped dramatically in Western Europe over the last decade, rates in the United States and the developing countries have remained high or have actually risen. In both settings, traditional prevention strategies are not working. Therefore, it is reasonable to readdress an old idea: antibiotic treatment on a community-wide basis in high risk settings. Selective mass treatment has been successful in the past, at least with a few diseases. Examples are the campaigns against yaws in Africa and endemic treponematosis in Yugoslavia in the 1950's. Monthly treatment of prostitutes in Indonesia dramatically lowered the syphilis seroprevalence rates in that country. On the other hand, a country wide mass treatment strategy for the eradication of gonorrhea in Greenland failed. Incidence rates decreased significantly during and immediately following the campaign but rapidly climbed back to previous levels soon thereafter. This experience suggests that diseases with relatively low infectivity would be most amenable to control by mass treatment. Compared to the gonococcus (GC), *Chlamydia trachomatis* (Ct) is transmitted less efficiently and therefore may be a reasonable candidate for control through mass treatment. Studies are need to test this hypothesis. The effect of mass treatment on chancroid is more difficult to predict. Considerations in determining which diseases might be approached by a mass treatment strategy would include the following: The target disease should have high morbidity and/or have a significant role in enhancing HIV transmission. It should have a high prevalence in the target population, and it should be susceptible to treatment by a safe oral antibiotic given as a single dose. Experimental trials of mass treatment must measure the following: emergence of resistance of both the target organism and other human pathogens, population effects of drug related adverse events including the special problems associated with unrecognized pregnancy, and effects on sexual risk-taking behavior. Additionally, there are ethical issues. The mass treatment approach assumes that benefits of the program to society outweigh the effects of drug related adverse events on the individual. This may not always be true. In the U.S. selective mass treatment might stigmatize certain ethnic groups such as African-Americans. Are decreasing STD incidence rates in these groups worth it? There are a number of pragmatic problems with mass treatment in the U.S. such as cost, political resistance to using health care dollars to combat what some would consider to be a moral not public health problem, and our medical legal system. For these reasons, plus the fact that in this country widespread screening strategies for Ct and GC soon may be economically feasible, it seems unlikely that large scale mass treatment strategies could ever be done here. In conclusion, data is needed on disease specific efficacy of mass treatment strategies and the population effects of drug related adverse events. With such data in hand, decision analyses could be applied using variables from a variety of different settings in order to determine the cost effectiveness of mass treatment strategies for STD control worldwide.

D5-018 BEHAVIORAL CHANGE AND STDS. Heather G. Miller[1,3], Jonathan Zenilman[2], and Charles F. Turner[3] [1]STD Branch, NIAID, Bethesda, [2]Johns Hopkins University, Baltimore, [3]Research Triangle Institute, Rockville, MD

A number of sexual and health-related behaviors have been shown to be associated with the prevention and transmission of STDs. Despite nearly a decade of HIV-related intervention research, there remain questions about whether or not behavior can be changed. Data will be presented from both national probability surveys and from several smaller, focused intervention studies that support the hypothesis that rates of sexual risk taking and condom use change over time. While such data are vital in evaluating interventions and assessing background rates of risk taking, there also remain questions concerning their interpretation. Does increased reporting of condom use reflect more use or more reporting of use? Are respondents telling the truth? Do subjects understand the meaning of the questions being posed by investigators? Does the context in which measurements made affect data quality? Data will be presented to probe nonsampling error in measures of sensitive behavior, including comparisons of data collected under different conditions and independent corroboration of the validity of self-reported sexual behaviors. Finally, research needs to improve understanding for factors that shape behavior change as well as bias in reporting will be discussed.

Vaccines and STDs I & II

D5-019 DEVELOPMENT AND TESTING OF PROPHYLACTIC AND THERAPEUTIC VACCINES FOR GENITAL HERPES, Stephen E. Straus, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

Widespread application of antiviral chemotherapy has diminished the physical and psychosocial impact of genital herpes, but it is not a strategy that it is appropriate for or acceptable to all infected individuals. More importantly, antiviral drugs have no definite impact on the spread of HSV itself. These issues, coupled with the potential for progressive resistance of virus to existing drugs, necessitates serious exploration of vaccines for prevention and even treatment of genital herpes. Over the past decades many attempts were made to induce or boost immune responses to HSV by using unrelated vaccines, killed HSV, or partially purified proteins. Unfortunately, none has proven effective under the scrutiny of a well-controlled study.

Within the past several years more contemporary technologies have been exploited for HSV vaccine development. Two of the approaches have already led to candidate vaccines that entered clinical trials. Rationally engineered live recombinant HSV in which some known virulence sequences were deleted proved too highly attenuated to induce substantial immune responses. Attempts are underway to re-engineer the vaccines using more focused deletions and mutations.

Phase II and III studies are already in progress using recombinant HSV glycoproteins. A series of exploratory studies documented that glycoproteins B (gB2) or D (gD2) alone or in combination are able to induce substantive primary cellular and neutralizing antibody responses. When given in the context of a novel lipid adjuvant, such as MF59 developed by Biocine, primary responses to HSV could be elicited that exceed levels seen in infected individuals. gB2, gD2, and MF59 vaccines are under study for prevention of genital herpes. A pilot study using gD2 in the traditional alum adjuvant proved able to reduce rates of genital recurrences in previously infected people by 30%. More extensive immunotherapeutic studies are underway using the gB2, gD2, and MF59 vaccines. SmithKline Beecham is exploring similar types of vaccines using gD2 alone.

By the end of this decade we will know whether one or more of the vaccine candidates is able to effectively treat or prevent genital herpes.

Sexually Transmitted Diseases in the HIV Era

D5-100 SERO-EPIDEMIOLOGIC FIELD EVALUATION OF AN ENZYME IMMUNOASSAY (EIA) FOR *HAEMOPHILUS DUCREYI* ANTIBODY.

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Strategy for HIV control includes control of *H. ducreyi* through public health STD intervention, as chancroid is a major promoter of HIV dissemination. Sero-epidemiologic identification and measurement of *H. ducreyi* antibody would permit targeting and evaluation of STD control efforts upon chancroid. We evaluated a proposed sero-epidemiologic application of the *H. ducreyi* antibody EIA in the setting of the Ministry of Health AIDS Control Program and national STD reportage in Costa Rica. During 1986 - 91, there has been a sustained, stepwise fall in annual population incidence of chancroid from 1014 to 101 cases, or 3.7 to 0.3 cases/10,000. In the National Sentinel HIV Seroprevalence Survey, we evaluated HIV-1, HIV-2 and *H. ducreyi* antibody seroprevalence in serial serosurveys of urban prostitutes and central urban public STD clinic clientele:

	Jan - Mar 1991		Oct - Dec 1992	
	STD Clinic	Prostitutes	STD Clinic	Prostitutes
HIV neg.	397	415	394	494
H.ducreyi +	36 (9.1%)	14 (3.1%)	14 (3.5%)	32 (6.3%)

The statistically significant decrease in *H. ducreyi* antibody seroprevalence in STD clinic clientele corroborates the substantial preceding fall in national reportage of chancroid. The increased seroprevalence in women working as prostitutes is of concern, and may warrant targeted STD evaluation and intervention for HIV prevention.

D5-101 SECULAR TRENDS IN SEXUALLY TRANSMITTED DISEASES IN DENVER, CO., 1978-93. Susan P. Dias, John M. Douglas, and Franklyn N. Judson. Denver Public Health, Denver, CO.

Trends in STD prevalence rates among patients attending an urban STD clinic were assessed for the 16 year period of 1978-1993. Two year prevalence rates for each disease were determined by dividing new cases by patient visits. Visits by heterosexual men (HM) fluctuated over 16 years but were overall similiar while visits by gay men (GM) declined by 72% and those for women (W) increased by 30%.

		78-79	80-81	82-83	84-85	86-87	88-89	90-91	92-93
Visits	HM	21514	22387	20770	20655	21398	19463	19830	21132
	GM	11037	15348	17022	11589	4958	3584	3623	3135
	W	10994	11591	11560	11859	12372	11363	12935	14323
GC	HM(%)	15.4	14.2	12.2	13.9	10.9	8.2	8.0	10.0
	GM(%)	24.3	22.1	17.1	14.5	8.8	5.1	4.3	4.4
	W(%)	18.7	15.1	11.4	12.4	8.4	7.1	6.9	7.8
Syph	HM(%)	0.4	0.4	0.4	0.6	0.6	0.5	0.5	0.6
	GM(%)	1.5	2.0	1.4	1.3	2.1	1.1	1.3	1.0
	W(%)	0.6	0.3	0.5	0.4	0.4	0.6	0.7	0.6
Trich	W (%)	10.9	10.1	8.9	9.5	8.2	6.7	6.1	5.8
NGU	M(%)	25.4	22.7	18.9	19.4	21.1	21.7	25.2	24.7
Herpes	M(%)	2.4	2.2	2.8	3.5	3.6	3.4	2.4	2.5
	W(%	2.0	2.2	3.5	4.3	3.5	2.6	3.4	2.0
Warts	M(%)	6.6	8.5	9.9	11.6	16.3	18.9	17.5	15.1
	W(%)	4.1	5.9	5.9	6.1	10.1	12.2	8.6	7.7

GC prevalence declined from 78-79 to 92-93 by 35%, 82%, and 58% among HM, GM, and W, respectively. The largest decline occurred between 84-85 and 86-87 for all groups with rates further decreasing by 50% for GM, and stable for HM and W. For syphilis, rates generally declined for GM (33% overall), but showed little variation for HM and W. The trichomonas trend paralleled that of GC among W with a decline of 47% over 16 years. NGU prevalence fluctuated from 78-79 to 92-93 but changed minimally; rates decreased by 26% from 78-79 to 82-83 and then rose progressively through 90-91. Herpes prevalence peaked in the mid 1980s for both M and W but varied minimally from 78-79 to 92-93. In contrast, genital warts increased both among M and W, with rates over the 16 year period rising by 129% for M and 89% for W. Secular trends in the prevalence and incidence of specific STDs will be discussed in relationship to period sexual behavior modifiers including the AIDs epidemic.

D5-102 THE LPS FROM *CHLAMYDIA TRACHOMATIS* IS THE MAJOR DETERMINANT OF THE ACUTE INFLAMMATORY RESPONSE TO INFECTION. R.R. Ingalls[1], P.A. Rice[1], N. Qureshi[2], K. Takayama[2], J.L. Lin[1], and D.T. Golenbock[1]. [1]The Maxwell Finland Lab. for Infect. Diseases & Boston Univ. School of Med., Boston, MA, 02118; [2]Middleton V.A. Hospital, Madison, WI, 53705.

Chlamydia trachomatis (*CT*) is a major etiologic agent of sexually transmitted diseases in the United States. Chronic infections with *Chlamydia* are common and may be important in the pathogenesis of infertility, as well as the transmission of other STDs, including HIV. Infection with *CT* is followed by an inflammatory response involving phagocytes. The molecular characterization of this acute immune response to *CT*, especially with respect to the role of its lipopolysaccharide (LPS), remains to be undertaken. 15 mg of LPS were prepared by hot-phenol extraction from 5 x 10^{12} *CT* elementary bodies (EB). Monophosphoryl lipid A (*CT* MPLA) was prepared, methylated, and purified by reverse-phase HPLC for structural analysis. The majority of the lipid A (~ 70%) was found to be pentaacyl. When the pentaacyl *CT* MPLA was subjected to mass spectrometry, molecular ions were observed at m/z 1900-2000. These data are consistent with the presence of long-chain fatty acids in the lipid A moiety but suggest much less structural heterogeneity than has previously been reported (Infect. Immun. 48:573, 1985). The endotoxin activities of whole *CT* EB and purified LPS were characterized in comparison to whole *Salmonella minnesota* R595, *S. minnesota* ReLPS and lipooligosaccharide from *Neisseria gonorrhea* (*GC* LOS). *CT* LPS induced the release of TNF-α from whole blood *ex vivo* but was ~100-fold less potent when compared to ReLPS and *GC* LOS. Similarly, whole EB were ~100-fold less effective in inducing TNF-α release when compared to intact *Salmonella*. Specific LPS antagonists, such as *Rhodobacter sphaeroides* lipid A and lipid IV_A (J. Biol. Chem. 266: 19490, 1991), inhibited both *CT* EB and *CT* LPS-stimulated TNF-α release. Both *CT* EB and *CT* LPS were capable of inducing the translocation of nuclear factor-κB in a Chinese hamster ovary fibroblast cell line transfected with the LPS receptor, CD14. These data suggest that chlamydia infection can activate the immune system, as evidenced by the generation of cytokines and *CT*-related NF-κB translocation in response to whole EB, and that the effects are mediated through LPS. The data also provide an *in vitro* explanation for the clinical observation that *CT* is a less potent inducer of the inflammatory response than infections with other Gram-negative organisms.

D5-103 MURINE SEROLOGICAL RESPONSES TO PREDEFINED *CHLAMYDIA TRACHOMATIS* ANTIGENS, Claudia J. Lammel, Qixun Zhao, and Richard S. Stephens, Department of Laboratory Medicine, University of California, San Francisco, CA 94143 and the Program in Infectious Diseases, University of California, Berkeley, CA 94720

Serological responses elicited following *C. trachomatis* infection have been associated with immune protection by antibodies that are serovariant-specific and targeted to the major outer membrane protein (MOMP). The relationship of immune neutralization by antibodies specific to MOMP antigens that are serovar-specific and the presence of antigenic variation for these antigens suggest that antigenic variation is a mechanism by which *C. trachomatis* evades host immune responses that are elicited following infection. There are a wide variety of serovars that generally fall within B-, C-, or G-serocomplexes because of antigenic and amino acid sequence similarities. We modeled the effect on the serological immune response to reinfection of susceptible hosts by different serovars in a murine model. Groups of mice were infected with serovars A, B, C, D, G, and I. These serovars represent two from the B-complex serogroup, one from the intermediate G serogroup, and three from the C-complex; each have different serological relatedness within the serogroups. One year after the initial infection subgroups of primed mice were challenged with the homologous or each of the heterologous serovars. The serological specificity was evaluated using the sequence-defined immunoassay. Each group produced antibodies to VS1 except those primed with serovar G. Only five of the 36 groups had serological reactivity to VS2 peptides. Several conclusions can be tentatively drawn. First, there was a recall response of the initial serovar following infection by a heterologous serovar, but only among serovars related by serogroup and not across serogroups. Second, a few pairs led to some level of apparent cross-reactivity for a particular VS1 peptide, but only between closely related serovars. Third, the serovar specificity for the challenge was readily detected. One exception was the boosted reactivity to serovar G-primed mice that showed little VS1 reactivity despite a boosted response to OMP2. The murine challenge data strongly suggest that monitoring sequential sera from an individual for VS1 peptide-specific responses will be capable of detecting responses indicative of subsequent infection by a different serovar and also detect previous infection by a different serovar.

D5-104 ASSOCIATION OF KS WITH A LATENT SEXUALLY-TRANSMITTED AGENT, Leslie G. Louie[1], Susan Desmond-Hellmann[2], Nicholas Hellmann [2], Alex Kizito-Muyombya[2], Edward Katongole-Mbidde[2], and Ira B. Tager[1,2]. [1]School of Public Health, Univ. California, Berkeley, CA, 94720. [2]Makerere Univ./Univ. California at San Francisco Collaborative Health Project, Kampala, Uganda.

This study was undertaken to test the hypothesis that Kaposi's Sarcoma (KS) may be caused by a sexually-transmitted agent(s). Cases are 345 men with KS who presented to the Uganda Cancer Institute between May 1989 and August 1991. Controls are 783 men from the Mulago Hospital Sexually Transmitted Disease (STD) Clinic who reported no general symptoms of illness (e.g., lymphadenopathy, weight loss, sweats, diarrhea, rash). Demographic information, sexual history, history of STD-related symptoms (genital ulcers and penile discharge), and HIV-1 serology were collected from all subjects. Report of ulcers and discharge are further stratified by timing of the event: "recent" (within the past year) and "old" (>2 years ago). HIV-1 seropositivity is the greatest risk factor for KS (OR=12.61, p<0.0001). The effects of HIV-1 on KS are modified by timing of symptoms: "recent" ulcers, OR=58.58; "old" ulcers OR=11.13; "recent" discharge, OR=52.34; and "old" discharge, OR=11.71 (p<0.0001) for the occurrence of HIV-1 among KS cases vs. STD controls. Therefore, further analyses are stratified by HIV-1 status.

Among HIV(-) males, "recent" ulcers and discharge are less prevalent among KS cases vs. controls (ORs = 0.07 and 0.02, respectively) whereas "old" ulcers and discharge are more prevalent among cases (ORs = 3.58 and 4.00, respectively). Among HIV(+) subjects, the comparable odds ratios are 0.33, 0.10, 3.23, and 3.52, respectively (p<0.0001). Among KS subjects, the proportion with HIV-1 is higher for subjects reporting "recent" ulcers (OR=7.01, p=0.004) but not for "old" ulcers or any category of discharge. These data indicate that the etiology of KS is heterogeneous, with some cases due to latent effects of a sexually-transmitted agent(s) that initially presents as a genital ulcer, and other cases caused by as yet undetermined factors. In the presence of HIV-1, the infection may result in KS after a shorter period of time, supporting the synergy hypothesis (Ensoli et al., Nature 1994; 371:674).

D5-105 INVESTIGATION OF PAST AND FUTURE CANDIDATE GONOCOCCAL VACCINES. Peter A. Rice, Daniel P. McQuillen, Sunita Gulati, Milan S. Blake, Edward W. Hook, Richard S. Kaslow, Peter K. Kohl, and Thomas M. Buchanan. Maxwell Finland Lab for Infectious Diseases, Boston City Hospital, Boston MA 02118

In 1985, a placebo-controlled vaccine trial was conducted at the University of Washington that used a prototype vaccine (prepared from gonococcal strain NRL 5767) that was enriched with porin protein (Por — approximately 85%), but also contained reduction-modifiable protein (Rmp — approximately 15%) and small amounts of lipooligosaccharide (LOS). Overall, the vaccine had no effect in preventing the onset of experimental infection. Following intraurethral challenge with live homologous gonococci, 18/35 (51%) of vaccine and 19/27 (68%) of placebo recipients became infected (p=NS). Serum antibody (Ab) levels were measured to the three major components (Por, LOS, Rmp) prepared from the challenge strain — before administration of vaccine (or placebo) and again just prior to intraurethral challenge. In a model that assumed interdependence of the three Ab levels, we used the ratio $\left(\frac{Por\cdot Ab + LOS\cdot Ab}{Rmp\cdot Ab}\right)$ to characterize a beneficial effect of Por and LOS antibodies and a subversive effect of Rmp antibodies. The level of this ratio just prior to challenge appeared to protect against infection when vaccination status and change in bactericidal activity were included in the model (p=0.01, Odds ratio = 6.1), but not when vaccination status was excluded. Monoclonal antibody (mAb) 2C7 recognizes a conserved LOS epitope expressed *in vivo* by 95% of gonococci. This epitope is a target of the immune response in patients with natural infection and in response to immunization with the porin vaccine described above. To characterize the immune response to the 2C7 gonococcal LOS epitope in an animal model, we immunized mice with mAb 2C7 (Ab1) and generated the anti-idiotypic mAb CA1 (Ab2). Immunization of mice and rabbits with mAb CA1 yielded Ab3 antibodies that possessed bactericidal activity, in the presence of homologous complement, against gonococci expressing the 2C7 epitope. Further, rabbit Ab3 antibodies mediated opsonophagocytosis of gonococci expressing the 2C7 epitope by human polymorphonuclear leukocytes, as measured in a flow cytometric assay. These data indicate that mAb CA1, acting as a surrogate for gonococcal LOS, may yield a candidate immunogen to serve as the basis for potentially protective vaccine.

D5-106 IMPLEMENTATION OF A COMMUNITY-LEVEL PROGRAM TO ENHANCE CHLAMYDIA AND GONORRHEA SCREENING AMONG URBAN MINORITY YOUTH IN DENVER, Cornelis A. Rietmeijer, Paul Z. Simons, Keith Yamaguchi, Charlene G. Ortiz, Sydney A. Montstream, Franklyn N. Judson, John M. Douglas. Denver Public Health, Denver CO.

Objective: To develop a community-level peer-based program among minority youth in Denver to enhance screening for C. trachomatis (CT) and N.gonorrhoeae (GC).

Methods: 1) A peer-volunteer network was developed to distribute and discuss safe-sex information, condoms, and STD screening vouchers. 2) Vouchers are redeemed at clinic-based sites for free comprehensive STD evaluations, including CT and GC screening, and a $5.00 incentive. 3) For men, vouchers can also be redeemed for urine CT testing by polymerase chain reaction (PCR) in community-based settings, including a public health storefront.

Results: 1) Since the implementation of the program in May 1994, a total of 165 peer volunteers have been trained, of which 60 (36%) have been retained $\geq$ 4 months. During this period, the volunteer network distributed 13,089 condoms and 1,074 screening vouchers. 2) Comprehensive clinic-based STD screening was performed on 22 clients (8 M, 14 F), yielding 5 cases of GC, 2 cases of CT, and 1 case of syphilis. Five clients had a variety of other STD, including molluscum contagiosum, genital warts, and pediculosis. Only 9 clients (41%) had no STD. 3) Community-based urine PCR screening for CT was implemented in August 1994. Through October, 3 (2.9%) of 104 men screened were CT+.

Conclusions: 1) Community-level peer-based STD programs can be implemented in a relatively short time. 2) Although the number of referrals for clinic-based screening has been limited thus far, the prevalence of STD in this group is high. 3) Barriers to STD screening services need to be further examined in these high risk populations and the feasibility of less invasive procedures such as urine testing in non clinic-based settings must be studied.

D5-107 YIELD OF PAP SMEAR SCREENING IN AN STD CLINIC SETTING. J. C. Shlay, W. L. McGill, H. A. Masloboeva, and J. M. Douglas, Denver Disease Control, Denver, CO

To assess the benefit of performing Pap smear screening in an STD clinic, we evaluated rates and risk factors for inadequate and abnormal smears among women screened from 1991-1994. Criteria for screening included no pap smear in the past year, history of an abnormal smear, genital warts on exam, or recent prostitution. Because chlamydia (CT) testing was not routinely performed on women with a condition warranting therapy, those with a diagnosis of gonorrhea (GC), mucopurulent cervicitis (MPC), or pelvic inflammatory disease as well as a positive CT test were considered to have a CT-associated diagnosis (CT-dx). Of 1992 smears collected, 209 (10.5%) were inadequate, 251 (12.6%) had atypia, 211 (10.6%) had low-grade squamous intraepithalial lesions (SIL), and 51 (2.6%) had high-grade SIL (HSIL). Inadequate smears were more common among women with trichomoniasis (16%, p=.02) and pregnancy (17%, p=.03), but not among those with GC (16%), MPC (10%), or all CT-dx (9%). Risk factors associated with all SIL were current genital warts (p<.001), abnormal cervix on exam (p<.01), younger age ($\leq$19, 21.1%; $\geq$35, 5.4%, p<.01), and CT-dx (p<.01). Factors associated with HSIL included history of herpes (p<.05) and genital warts (p<.01), but no currently diagnosed STD. LSIL was not consistently associated with number of lifetime sexual partners; HSIL increased with increasing partners for adolescents but not older women. Smears with SIL with evidence of human papillomavirus (HPV⊕) (i.e., koilocytotic atypia) were associated with younger age (p<.001), current genital warts (p<.001), and abnormal cervix on exam (p<0.05), while smears with HPV⊖ SIL were associated with current MPC, CT-dx and past GC (p<.05), but not past or current genital warts. In summary, Pap smear screening in an STD clinic setting is both feasible, with rates of inadequate smears of < 20% even with inflammatory STDs, and high-yield, with rates of abnormalities requiring further follow-up of > 20%. The association of CT-dx with HPV⊖ SIL supports the premise that CT as well as HPV maybe contribute to cervical neoplasia.

D5-108 CHLAMYDIA ARE RESISTANT TO TOXIC EFFECTS OF LYTIC PEPTIDES, William J. Todd[1,2], Johannes Storz[1], William G. Henk[3], Nell R. Morris[2], and Richard P. Morrison[4], Departments of Veterinary Microbiology and Parasitology[1], Veterinary Science[2], Veterinary Anatomy and Cell Biology[3], Louisiana State University, Baton Rouge, LA 70803, and The Rocky Mountain Laboratories[4], Hamilton MT 59840

Lytic peptides are small basic proteins usually consisting of only 18 to 39 amino acids. Following the pioneering work of Hans Bowman and coworkers, lytic peptides are now known to be important components of antimicrobial defence systems of many species including, insects, amphibians, and mammals. The killing effect of most lytic peptides is believed to reside in formation of an amphipathic alpha helix capable of disrupting membrane structure. Because some lytic peptides are known to kill many gram (-) and gram (+) bacteria at concentrations below toxicity for normal eukaryotes, we investigated the ability of several lytic peptides to preferentially kill both chlamydial infected cells, and cell-free infectious elementary bodies. Cecropin, synthesized analogs of cecropin, and melittin were used. Hela cells were infected with the serovar D strain of venereal-disease causing chlamydia. At 24 and 48 hours post infection, the infected cells and non-infected controls were incubated with various lytic peptides. Selective killing of the chlamydial-infected cells was not detected. Likewise, when elementary bodies from a sheep abortion strain of chlamydia were incubated with lytic peptides prior to infection of cultured cells, only minimal reduction in infectivity occurred even at peptide concentrations several fold higher than required to kill free growing gram (-) and gram (+) bacteria. The elementary bodies were relatively resistant to the effects of the lytic peptides tested. The possibility of using lytic peptides to prevent or eliminate chlamydial infections does not appear to be promising.