BACTERIA - HOST CELL INTERACTION

Marcus Horwitz and Michael Lovett, Organizers
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Plenary Sessions Page

Poster Sessions

Host Bactericidal Mechanisms

H 001 BACTERICIDAL/PERMEABILITY INCREASING PROTEIN (BPI) OF GRANULOCYTES: STRUCTURE AND FUNCTION, Peter Elsbach, NYU Medical Center, New York, NY 10016.

Cytotoxic proteins (peptides) have served prokaryotic, plant and eukaryotic cells throughout evolution to kill other cells. Some of these cytotoxins are highly selective in their action, others can kill or damage non-specifically. BPI isolated from the primary granules of human or rabbit granulocytes kills only gram-negative bacteria and is non-toxic for other cells. The highly basic human and rabbit BPI (M_r 58 and 50 Kd respectively) are closely similar proteins as judged by their amino acid composition and NH_2-terminal sequence and the presence of common antigenic determinants. The two proteins occur only in cells of the myeloid series and have similar bactericidal range and potency. The target cell specificity of BPI for gram-negative bacteria is explained by a strong affinity for LPS that is mediated by charge-charge and hydrophobic interactions. Surface-binding of BPI causes: 1) irreversible loss of colony formation; 2) permeabilization of the outer membrane for normally impermeant hydrophobic substances; 3) selective activation of bacterial phospholipid and peptidoglycan degradative enzymes, and 4) cessation of synthesis of outer membrane pore protein F and stimulation of synthesis of porin C, with minimal inhibition of overall protein synthesis. Addition of 80 mM Mg^{2+} or Ca^{2+} or trypsin to BPI-killed bacteria induces repair of the outer membrane permeability barrier by synthesis of new LPS. Thus, BPI produces a highly discrete lethal lesion. BPI-sensitive bacteria are killed effectively by O_2-deprived granulocytes or granulocytes unable to carry out the respiratory burst (CGD). Limited proteolysis with purified human granulocyte elastase produces 4 main fragments. Digests that reveal little or no native protein on SDS-PAGE show no fragmentation on PAGE under non-reducing, non-denaturing conditions and retain full biological activities, indicating that intramolecular forces keep fragments together across the few sites that are susceptible to proteolytic cleavage. These observations resemble those made on bacterial toxins and suggest that the molecular organization of this mammalian cytotoxin had its origin early in evolution.

H 002 DEFENSINS- ANTIMICROBIAL AND CYTOTOXIC PEPTIDES OF PHAGOCYTES, Tomas Ganz, Department of Medicine, University of California, Los Angeles, CA 90024.

Human neutrophils contain three antimicrobial peptides, named defensins, with in vitro activity against a variety of bacteria, fungi and enveloped viruses. The peptides are cytotoxic to several neoplastic cell lines as well as human lung fibroblasts. The three human defensins are members of a family of arginine-rich peptides, 29-34 amino acids in length, all of which contain six homologous disulfide-bonded cysteines, as well as other homologous amino acids. Other members of the defensin family are principal granule constituents of rabbit and guinea pig granulocytes and rabbit alveolar macrophages. Defensins make up about a third of the total protein content of azurophil granules of human PMN and are especially plentiful in the largest and densest azurophil granules. Defensins account for nearly all of the microbicidal activity of human PMN granule extract against the model bacterium Acinetobacter calcoaceticus HO-1. The activity of defensins against bacteria is potentiated by certain metabolic substrates and competitively inhibited by serum proteins and increasing ionic strength. Although defensins are released by phagocytosing PMN both into their phagolysosomes and externally, the peptides are more likely to reach cidal concentrations in the confined spaces of phagolysosomes or in intercellular clefts. Along with other microbicidal proteins and reactive oxygen intermediates defensins may generate a broad spectrum antimicrobial environment in PMN phagolysosomes.

H 003 MECHANISMS OF BACTERIAL RESISTANCE TO COMPLEMENT-MEDIATED KILLING, Keith A. Joiner, NIH, Bethesda MD 20814.

Gram negative bacteria utilize a variety of mechanisms for evasion of direct killing by the cytolytic C5b-9 terminal attack complex of complement. These include inefficient complement activation, a block in the cascade after initial activation, or failure of the formed C5b-9 complex to insert effectively into the outer membrane. We have shown previously that a serum resistant strain of Salmonella bearing O Antigen polysaccharide (O-Ag) in its LPS activates complement efficiently; however the C5b-9 complex which forms does not insert into hydrophobic domains of the outer membrane, is shed and is not bactericidal. To investigate this further, we used a mutant strain of Salmonella montevideo requiring exogenous mannose (M) for O-Ag synthesis. When grown in optimal concentrations of M, the O-Ag is composed of from 0 to more than 55 subunits, each containing 4 M residues together with glucose and n-acetyl glucosamine. LPS was prepared from these cells grown under 3 different conditions where the availability of exogeneous M was regulated such that the average number of O-Ag units/LPS molecule, the percent of LPS molecules bearing long O-Ag side chains, and the % of lipid A cores bearing O-Ag were all varied. LPS profiles were monitored on SDS-PAGE and cells with different LPS profiles were tested for serum sensitivity. Survival in serum was associated with LPS that contained an average of 4-5 O-Ag units/LPS molecule and 20 to 23% of the LPS molecules with >14 O-Ag units/LPS molecule. Serum survival was less clearly associated with the % of lipid A cores covered with O-Ag. We propose that the O-Ag provides protection from serum killing by sterically hindering access of the C5b-9 complex to the outer membrane and that a critical density of long O-Ag is necessary for protection.

H 004 HUMORAL AND CELLULAR IMMUNE MECHANISMS POTENTIALLY OPERATIVE IN THE PATHOGENESIS OF SYPHILIS, James N. Miller and Michael A. Lovett, UCLA, Los Angeles, CA 90024.

During the past decade, the demonstration in this laboratory of 1) specific T. pallidum-host cell attachment (Fitzgerald), 2) complement-mediated treponemicidal activity of non-immune and immune sera (Bishop, Blanco, Hanff), and 3) in vitro phagocytosis by rabbit peritoneal macrophages (Lukehart), has provided important functional approaches to our understanding of the immunological sequence of events following host-treponeme interaction in both the human and experimental disease. Application and/or extension of these functional assays in several laboratories, together with the use of molecular strategies, have resulted in both suggestive and compelling data implicating humoral and/or cellular immune mechanisms in the initiation, proliferation, healing, and exacerbation of the disease process and will be discussed in depth.

Although the role of specific surface and subsurface components of T. pallidum in the immunopathogenesis of syphilis has been an enigma, recent and continuing structure-function studies from the Lovett and Miller laboratories utilizing recombinant surface and native endoflagellar antigens have revealed an outer membrane with unique properties relating to antibody surface binding and location of endoflagellar antigens. Further studies on the structural and functional characterization of these antigens and their relationship to immunopathogenesis will also be discussed in detail.

H 005 MYELOPEROXIDASE-MEDIATED DAMAGE TO THE E. COLI RESPIRATORY CHAIN: A MECHANISM OF ANTIMICROBIAL ACTIVITY?, Henry Rosen, Ann M. Waltersdorph and Seymour J. Klebanoff, Department of Medicine, University of Washington, Seattle, WA 98195. Among the diverse antimicrobial systems available to the polymorphonuclear phagocyte (PMN) is one that consists of PMN myeloperoxidase (MPO), PMN-derived H_2O_2, and an oxidizable halide cofactor such as Cl^-. The cell-free MPO+H_2O_2+Cl^- system produces HOCl (among other antimicrobial oxidants). The bacterial cytoplasmic membrane, a prime candidate site for lethal MPO-mediated oxidations, contains multiple oxidizable proteins that mediate the transport of nutrients and their conversion, through an electron transport chain, to metabolic energy. Reagent HOCl rapidly impairs membrane transport of nutrients and, later, destroys cytochrome b and stops respiration (Albrich MJ et al, PNAS 78:210;1981, FEBS Lett 144:157;1982, J Clin Invest 78:177;1986). The MPO+H_2O_2+Cl^- system also attacks the E. coli membrane. Iron-sulfur clusters in membrane proteins are rapidly destroyed and microbial iron slowly leaks into the suspension medium. In contrast to reagent HOCl systems, loss of E. coli respiration is an early event in MPO-mediated killing. Under appropriate growth conditions the respiratory chain in E. coli is essentially linear as follows: substrates $\longrightarrow$ dehydrogenases $\rightarrow$ ubiquinone $\longrightarrow$ cytochrome b $\longrightarrow$ $\longrightarrow$ terminal oxidase $\longrightarrow$ O_2. Cessation of respiration cannot readily be attributed to impaired cell uptake of substrate (succinate) which is diminished by only 50% when O_2 consumption is undetectable (<1% of initial activity). Activity of the iron-sulfur protein, succinate dehydrogenase (SDH), rapidly becomes undetectable suggesting this dehydrogenase is an early site of MPO-mediated attack. Total cytochrome b, measured by difference spectroscopy, is constant but substrate (succinate or glucose) induced cytochrome reduction is progressively slowed and ultimately abolished (<<1% of initial activity). Once reduced, cytochrome b is rapidly reoxidized by O_2 suggesting relative integrity of the distal portions of the electron transport chain. Conclusions are that MPO-mediated killing of E. coli is associated with early and progressive damage to the aerobic electron transport chain and that SDH is an early target of oxidant attack. Other dehydrogenases and ubiquinone have not been excluded as additional targets but cytochrome b and the more distal components of the chain appear to be relatively spared. Impairment of electron transport, if broadly based, is a likely mechanism for MPO-mediated microbicidal activity.

Adherence of Bacteria to Host Cells

H 006 INTIMACY OF THE SYPHILIS SPIROCHETE-HOST CELL INTERACTION, Joel B. Baseman and John F. Alderete, Department of Microbiology, The University of Texas Health Science Center, San Antonio, TX 78284.

Treponema pallidum, the etiological agent of syphilis, displays a tip-oriented adherence to eucaryotic cells and to the extracellular matrix (ECM). This highly polar tropism is time and temperature dependent, and metabolic integrity of the spirochetes is a requirement for successful parasitism. Non-pathogenic treponemes fail to adhere, suggesting a relationship between adherence and virulence. The specificity of the recognition event between T. pallidum and the host is mediated by three outer membrane treponemal proteins (adhesins of 89kDa, 37kDa and 32kDa) and host fibronectin (Fn). It appears that the cell-binding domain of Fn and especially the L-arginyl-L-glycyl-L-aspartic acid tripeptide responsible for eucaryotic cell attachment and spreading is the unique target for treponemal adherence. The immunogenic and chemical properties of treponemal adhesins, their expression in recombinant Escherichia coli, and the properties of the adherence event, such as selective tropism of treponemes to Fn in ECM rather than to host cell surfaces, will be discussed in terms of the nature and pathophysiology of syphilis. In addition, other surface properties of T. pallidum will be described, including the major immunodominant protein designated P6 (45kDa) and the observed in situ biological heterogeneity of treponemes.

H 007 MOLECULAR MECHANISMS OF THE INTERACTION OF GROUP A STREPTOCOCCI WITH ANIMAL CELLS. E.H. Beachey. VA Medical Center and University of Tennessee, Memphis 38104.

Increasing evidence has accumulated to support the idea that lipoteichoic acid (LTA) on the surface of group A streptococci mediates the attachment of the bacteria to buccal epithelial cells. The negatively charged polyglycerolphosphate backbone of LTA forms ionic bonds with clusters of positive charges on the bacterial surface macromolecules such as the a-helical coiled-coil structures of M protein to form the fibrous layer of the streptococcal cell surface and to orient the glycolipid ends of LTA toward the surface in such a way that they can interact with receptors or animal cells. The receptors on buccal mucosal cells appear to reside in fibronectin molecules bound to the mucosal cells. Both endogenous fibronectin already bound to the cells and exogenous fibronectin used to coat isolated epithelial cells promote the attachment of the organisms, whereas soluble fibronectin or its LTA sensitive streptococcal binding fragments block attachment. Absorption and elution studies of enzymatically fragmented fibronectin molecules has localized a hydrophobic LTA binding site at the NH_2-terminal region of the molecule. The location was confirmed with polyclonal antibodies against synthetic NH_2-terminal peptides of fibronectin. Certain monoclonal antibodies against the NH_2-terminal end of fibronectin block the binding of LTA and streptococci to immobilized fibronectin. Because LTA is masked by serum and tissue components such as albumin and fibronectin, recognition of streptococci by phagocytic cells requires complement (C3) bound to the surface of the organisms. M-protein rich streptococci mask their C3 receptors by binding fibrin(ogen), and thereby escape recognition. The recognition of M-rich streptococci by the phagocytic cells is dependent on the development of antibody against epitopes of M protein not masked by fibrin(ogen). Thus, LTA in complex with surface molecules such as M protein mediate the attachment to an NH_2-terminal lipid binding site(s) of the fibronectin molecule on mucosal epithelial cells. After invasion into deeper tissue, M-rich streptococci binds fibrin(ogen) and thereby evade C3-mediated recognition by phagocytic cells; the C3-blocking effect of fibrinogen is overcome by immune responses toward epitopes of the M protein not masked by fibrin(ogen).

H 008 A COMPARISON OF MICROBIAL INVASION STRATEGIES
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An overly simplistic view of microbial pathogenesis would be that pathogenic microrganisms can cause disease by adherence and growth on the surface of host cells or by adherence followed by direct entry into host cells. We may sometimes overlook the fact that microorganisms may have several distinct adherence factors which play a role in the pathogenesis of infection. In the case of Yersinia entrocolitica, for example, there are no less than three distinct cellular factors which provide firm attachment of the microorganism to the surface of eucaryotic cells, two of which are also associated with the subsequent direct entry of bacteria into host tissue. Similarly, the study of direct entry of bacteria into mamallian cells has focused on only a few cultured cell lines. The extension of such investigations has permitted the identification of differing modes of intracellular growth by pathogens depending on the nature of the eucaryotic cell line under investigation.

H 009 GENETICS OF VARIABLE OUTER MEMBRANE PROTEINS OF THE PATHOGENIC NEISSERIA, Terry D. Connell, D. S. Barritt, T. H. Kawula, G. L. Murphy, E. L. Aho, and J. G. Cannon, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27514.

The pathogenic Neisseria (N. gonorrhoeae and N. meningitidis) are notable for the extensive variability in surface antigens that they display. This variability occurs among different strains, as well as within variants of a single gonococcal or meningococcal strain. We have been studying two families of outer membrane proteins that demonstrate such variation. Both the Protein II (P.II) proteins of the gonococcus and the similar class 5 (C.5) proteins of the meningococcus show phase and antigenic variation, with reversible switches in expression state occurring at high frequency. Although the family of P.II proteins made by one gonococcal strain shows extensive structural homology, the proteins do bear unique antigenic determinants, as demonstrated by the binding of a panel of P.II-specific monoclonal antibodies. Using genetic transformation studies and cloning of P.II genes, we have shown that there is a structural difference in sequences encoding P.II proteins when the genes are "on" and "off." These differences do not involve major chromosomal rearrangements, but probably involve specific changes in an unusual signal sequence. All P.II genes that we have cloned and sequenced represent ones that were "off" in the gonococcal chromosome. All contain an unusual element consisting of different numbers of repeats of a pentameric unit (CTTCT), which appears to encode a translated signal sequence. In each of these genes, the ATG initiation codon is not in the same translational frame as the codons specifying the N-terminal amino acids of mature P.II protein. We believe that "off" P.II genes have undergone a structural change, possibly involving the CTTCT sequence element, which results in a frame shift and premature termination of the synthesis of P.II protein. This model is consistent with the observation that gonococci that are not producing any P.II protein do contain mRNA transcribed from P.II genes. In recent studies on meningococcal C.5 proteins, we have shown that this family of proteins also undergoes phase variation, and that C.5 genes also contain the repeated CTTCT element. The ability of both gonococci and meningococci to vary expression of these major cell surface antigens is probably important to their survival in different environments within the infected host.

H 010 GONOCOCCAL AND MENIGOCOCCAL IRON-AQUISITION MECHANISMS: POSSIBLE ROLES OF IRON-REPRESSIBLE OUTER MEMBRANE PROTEINS. D. Dyer, N. Carbonetti, W. McKenna, J. Adams, S. West, and P.F. Sparling, Depts. of Microbiology and Medicine, U. of N.C., Chapel Hill, N.C. 27514.

We are studying the mechanisms by which pathogenic Neisseria acquire essential iron from sources available *in vivo*, including lactoferrin (LF) the principle source of iron on mucosal surfaces and pus, and transferrin (TF) the principle source of iron in serum. Gonococci (GC) and meningococci (MC) differ from most bacteria in their lack of detectable soluble iron-chelators, or siderophores. However, both GC and MC can use the common enterobacterial hydroxamate siderophore aerobactin as an iron source. Genomic digests of GC showed specific hybridization to a cloned *E. coli* hydroxamate-utilization gene *fhuB*, but not to any other *E. coli* genes involved in synthesis, uptake or utilization of aerobactin. Using the phasmid vector λSE4, the gonococcal *fhuB* homologue was cloned and it complemented *E. coli fhuB* mutants. Thus, gonococci have retained a portion of the hydroxamate utilization system. The role of *fhuB* in GC iron acquistion and pathogenesis remains uncertain, pending isolation of *fhuB* mutants.

Using the antibiotic streptonigrin as a means of selectively killing iron-replete organisms, MC and GC mutants blocked in iron utilization have been isolated. One MC mutant is unable to transport iron from TF, LF, aerobactin, or inorganic sources, and is missing a 70K outer membrane Fe-repressible protein (FeRP). By Western blot, most patients with systemic MC or GC disease develop antibodies against the 70K FeRP (as well as many other FeRPs), and most strains possess an antigenically similar 70K FeRP. Other mutants are altered specifically in use of TF or LF as on iron source, but have no evident alteration of FeRPs by SDS-PAGE. Uptake of Fe from TF or LF is saturable at about 1 μM protein, and we hypothesize that there are specific receptors for TF andLF; the identity of the receptors is unclear. We have shown that polyphosphate is able to facilitate removal of Fe from TF, and since GC and MC contain abundant surface polyphospate, Fe may be removed from bound TF or LF by means of polyphosphate, and then transported into the cell by one or more of the FeRPs. The 70K FeRP is a commn pathogenic Neisseria antigen and may play an important role in Fe transport.

H 011 MOLECULAR APPROACHES TO UNDERSTANDING THE FUNCTION OF THE GROUP A STREPTOCOCCAL M PROTEIN: A UNIQUE VIRULENCE MOLECULE. V.A. Fischetti*, K.F. Jones*, S.K. Hollingshead#, and J.R. Scott#. *The Rockefeller University, New York, NY 10021 and #Department of Microbiology, Emory University, Atlanta, GA 30322.

M protein is a fibrillar molecule on the surface of the group A streptococcal cell which confers to the organism the ability to resist phagocytic attack. Physicochemical analysis of the molecule has revealed that it is composed of two alpha-helical chains combined to form a coiled-coil structure extending about 600 A from the cell surface. Sequence studies on the M gene has revealed 3 domains composed of sequence repeats within the N-terminal 2/3 as well as a membrane anchor and a cell wall stabilization region at the C-terminal end. From immunological studies combined with DNA probe analysis, regions within the C-terminal half of the protein appear to be conserved among other M serotypes while those in the N-terminal region vary among these types. Besides antigenic variation, M protein also varies in molecular size. M protein size mutants have been found to occur at a frequency of 1 in 2000 CFU in a laboratory grown strain. These variants have also been isolated in serial streptococcal isolates from the same patient as well as from different patients in outbreaks of streptococcal pharyngitis. Sequence analysis of the M gene from the size mutant strains revealed that size variation is due to homologous recombination events within the sequence repeat regions of the M molecule. It was found that such events, in some cases, results in a change in amino acid sequence. This mechanism may be responsible for the antigenic variation in M protein as well as other bacterial antigens.

Biology of Intracellular Pathogens

H 012 PROTECTIVE EPITOPES OF THE MAJOR OUTER MEMBRANE PROTEIN OF CHLAMYDIA TRACHOMATIS: IDENTIFICATION, FUNCTIONAL CHARACTERIZATION, AND MOLECULAR CLONING OF THE EPITOPES. Harlan D. Caldwell*, You-Xun Zhang*, Francis E. Nano*, Theresa Joseph* and Hugh Taylor+. Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT 59840* and The Wilmer Institute, Johns Hopkins University, Baltimore, MD 21205+.

We have employed a cynomolgus monkey model to investigate the immune response of Chlamydia trachomatis ocular infections. The objective of these studies was to identify surface antigens of C. trachomatis trachoma serovars (A, B, and C) that are potential candidate antigens for the development of recombinant subunit vaccine for trachoma. Protective immunity in the monkey model is homotypic and is believed to be mediated by lacrimal IgA antibody. We studied by immunoblotting analysis the temporal tear antibody response of monkeys experiencing a primary ocular infection with the trachoma serovar B to identify immunodominant antigens recognized by tear IgA antibodies that were specific for the infecting serovar. Tear IgA antibodies specific for a 68 kd, 60 kd, the major outer membrane protein (MOMP) and lipopolysaccharide (LPS) were identified. Tear IgA antibodies that recognized the MOMP were specific for the infecting B serovars MOMP, whereas antibodies reactive with LPS recognized this antigen in all trachoma serovars. These findings indicated that those epitopes that were unique to the B serovars MOMP and to which the host made secretory antibody may be protective.

To identify and more thoroughly characterize these putative protective epitopes, we generated a panel of murine monoclonal antibodies against the B serovars MOMP. Monoclonal antibodies that reacted with species-, subspecies-, and type-specific MOMP epitopes were identified by immunoblotting. Only those monoclonal antibodies that were specific for type and some subspecies-specific MOMP epitopes passively neutralized the in vivo toxicity of the B serovar for mice and neutralized the infectivity of the B serovar for the monkey eye.

Studies were undertaken to molecularly clone portions of the B MOMP gene that encoded for these protective epitopes. We have isolated several lambda gtll recombinants expressing antigens that are immunoreactive with protective monoclonal antibodies. Expression of these epitopes has been achieved as fusion products of MOMP polypeptide fragments to β-galactosidase. The recombinant fusion products are being used as immunogens to directly immunize mice against chlamydial toxicity to evaluate their protective ability.

H 013 INTRACELLULAR BIOLOGY OF LEGIONELLA PNEUMOPHILA. Marcus A. Horwitz, Nathaniel R. Payne, and Carolyn G. Bellinger-Kawahara, UCLA, Los Angeles, CA 90024

Legionella pneumophila, the agent of Legionnaires' disease, is a gram-negative facultative intracellular bacterial pathogen that multiplies in human monocytes and alveolar macrophages. L. pneumophila is phagocytized by an unusual mechanism termed coiling phagocytosis in which a phagocyte pseudopod is coiled around the bacterium as the organism is internalized. Phagocytosis is mediated by monocyte membrane antigens CR1 and CR3, the receptors for complement components C3b and C3bi, respectively; monoclonal antibodies against these receptors inhibit phagocytosis and intracellular multiplication. L. pneumophila fixes C3 to its surface, as demonstrated by enzyme-linked immunosorbant assay (ELISA) and Western blot analysis; C3 deposition is mediated by the alternative pathway of complement activation. Inside monocytes, L. pneumophila forms a specialized phagosome involving the sequential interaction of the phagosomal membrane with smooth vesicles, mitochondria, and ribosomes, and the bacterium multiplies within a ribosome-lined phagosome. The L. pneumophila phagosome does not fuse with monocyte lysosomes, as measured by acid phosphatase histochemistry or by prelabelling lysosomes with thorium dioxide. L. pneumophila inhibits phagosome acidification, as measured by quantitative fluorescence microscopy. The capacity of L. pneumophila to enter mononuclear phagocytes by a complement-dependent pathway, form a ribosome-lined phagosome, inhibit phagosome-lysosome fusion, and inhibit phagosome acidification may be critical to the bacterium's intracellular survival and replication. The L. pneumophila model has relevance to several other intracellular pathogens and is an excellent one for general studies of intracellular parasitism.

H 014 MACROPHAGES, HELPER T CELLS AND CYTOLYTIC T CELLS: POSSIBLE CONTRIBUTION TO HOST DEFENSE AGAINST MYCOBACTERIA, Stefan H.E. Kaufmann, Inge Flesch, Ingrid Müller, Shubhada Chiplunkar and Gennaro De Libero, Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG.

Acquired resistance against Mycobacterium tuberculosis and M.leprae, the etiologic agents of tuberculosis and leprosy, respectively, crucially depends on specific T lymphocytes and mononuclear phagocytes. The peripheral T-cell set can be divided into two major subsets: CD4 T cells preferentially activate target cells via lymphokines (e.g., activation of anti-microbial macrophage functions) and CD8 T cells preferentially lyse target cells via direct cell-cell contact (e.g., destruction of virus-infected host cells). Furthermore, it has become clear that mononuclear phagocytes are heterogeneous and differ in their anti-microbial potential: blood monocytes appear to have a higher anti-microbial potential than most resident tissue macrophages. In mice, CD4 or CD8 T cells were selectively depleted by injecting anti-L3T4 or anti-Lyt2 monoclonal antibodies, respectively. Afterwards these mice were infected with live M.tuberculosis organisms. Depletion of either subset resulted in significant increase in mycobacterial numbers indicating that both T cell subsets were required for effective anti-tuberculous protection. We have attempted to further analyze the contribution of CD4 T cells, CD8 T cells, and macrophages to host defence in the mouse system, using in vitro propagated T-cell lines and clones and bone marrow derived mononuclear phagocytes (BMMØ). CD4 T-cell lines with reactivity to M.tuberculosis or M.leprae, respectively, produced multiple lymphokines including interferon-γ (IFN-γ). T-cell lymphokines and IFN-γ were capable of activating tuberculostatic functions in BMMØ. Growth of M.bovis BCG Phipps and M.tuberculosis H37Rv was markedly inhibited whereas that of M.tuberculosis Middelburg was unaffected suggesting heterogeneity among M.tuberculosis strains towards IFN-γ activated macrophages. CD8 T-cell lines with reactivity to M.leprae or M.tuberculosis were able to lyse mycobacteria primed BMMØ. Interestingly, coculture of CD8 T cells with M.bovis infected BMMØ led to mycobacterial growth inhibition indicating that lysis of infected target cells has tuberculostatic effects. We propose that both helper and cytolytic T-cell functions contribute to acquired resistance against tuberculosis. Activation of macrophages with high antibacterial potential by T-cell lymphokines including IFN-γ results in mycobacterial growth inhibition. In contrast, macrophages with low antibacterial potential may not be able to affect intracellular mycobacteria after lymphokine activation. Mycobacterial growth may also be affected by lysis of infected target cells. Under less controlled conditions target cell lysis, however, may lead to bacterial dissemination and hence be detrimental for the host. In contrast, in a well organized microenvironment (e.g., granuloma) with helper T cells, cytolytic T cells, and fresh blood monocytes being present, lysis of infected tissue macrophages with low antibacterial potential could facilitate mycobacterial uptake by monocytes which then could be activated by lymphokines to express anti-bacterial functions.

H 015 MYCOBACTERIA: FACTORS RESPONSIBLE FOR MODULATING HOST RESPONSE; FACTS AND SPECULATION, Patrick J. Brennan, Dept. Microbiology, Colorado State University, Fort Collins, Colorado 80523.
Direct chemical analysis, immunoelectron microscopy, use of monoclonal antibody probes and cloned T cells, and molecular cloning of the genes of a few select species have resulted in definition of the surface antigens of several mycobacteria, obligate and opportunistic pathogen alike. All serovariants within the Mycobacterium avium-M. intracellulare complex are characterized by surfaces dominated by the species- and type-specific glycopeptidolipids with epitopes composed of highly unusual sugars. The surfaces of M. kansasii, M. szulgai, M. malmoense, etc., all opportunistic pathogens, are dominated by the extraordinary trehalose-containing amphipathic lipooligosaccharides, each endowed with a unique non-reducing oligoglycosyl end group. The surface of M. leprae and the electron transparent zone surrounding foci of infection are dominated by the unique phenolic glycolipid. Beneath these variable carbohydrate layers lies the peptidoglycan-mycolyl arabinogalactan complex, and permeating all of these is the highly antigenic lipoarabinomannan, anchored in phosphatidylinositol, the so-called lipoteichoic acid of Mycobacterium spp. Use of the lambda phage or cosmid expression vectors combined with monoclonal antibodies has allowed identification of the genes for a few select protein antigens of M. leprae and M. tuberculosis. Thus, mycobacteria have been the beneficiary of considerable activity concerning antigen definition. However, which of all of these candidate antigens are presented to T cells of the helper phenotype and which may be implicated in intracellular survival and persistence is a moot but intriguing question.

Virulence-Related Surface Structures - II. LPS and Capsules

H 016 LPS-INDUCED PROTEIN MYRISTOYLATION IN MACROPHAGES, Alan A. Aderem* and Zanvil A. Cohn, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 1021.

We recently demonstrated that LPS induces the acylation of 3 macrophage proteins with molecular mass of 68 kDa and a doublet of approximately 38-42 kDa (1). The fatty acid associated with each of the acylated proteins is myristic acid. The myristate is probably linked to the proteins through amide bonds, since it is not released by treatment with hydroxylamine. Palmitate, stearate and arachidonate are not incorporated into proteins in the same manner. LPS-induced myristoylation in macrophages is an early response and occurs maximally 30 min after exposure to 10 ng/ml LPS. Most of the myristoylated proteins are associated with the macrophage membrane fraction. The 68 kDa protein whose myristoylation is induced by LPS has been identified as a major protein kinase C substrate.

We have also reported that while LPS alone is not a good trigger of arachidonic acid (20:4) metabolism in macrophages it has the capacity to prime macrophages for greatly enhanced 20:4 metabolite secretion when the cells are subsequently challenged with a variety of triggers (2). The concentration and time dependence of LPS-induced myristoylation is consistent with a role for myristoylation in LPS-priming of the 20:4 cascade. We hypothesize that LPS primes macrophages for enhanced 20:4 metabolism by promoting the myristoylation of key proteins involved in the regulation of 20:4 metabolism. The myristoylation of these proteins promotes their association with membrane bound substrates and consequently, ensures more efficient catalysis during the mobilization and oxygenation of 20:4

1. Aderem, A.A., Keum, M.M., Pure, E., and Cohn, Z.A. (1986) Proc. Natl. Acad. Sci. USA 83:5817-5821.
2. Aderem, A.A., Cohen, D.S., Wright, S.D., and Cohn, Z.A. (1986) J. Exp. Med. 164:165-179.

H 017 THE ROLE OF THE HAEMOPHILUS INFLUENZAE TYPE B (HIB) CAPSULE IN THE HOST-PARASITE INTERACTION, Porter W. Anderson, Dept. Pediatrics, Univ. Rochester, NY 14642.

The Hib capsular polysaccharide (CP) is a polymer of (3-B-D ribose (1-1) ribitol-5-phosphate). It may be made with a terminal lipid postulated to be the basis for membrane attachment. The work of several labs has revealed a number of roles of the CP in pathogenesis: Mucosal colonization. The CP has an unclear role. The organization of genes for CP biosynthesis leads to loss of capsule expression at a frequency of about 0.1%, and the Cap variants appear to have a competitive advantage in the nasopharynx. Bloodstream invasiveness. Hib is resistant to antibody (Ab)-free serum, and the CP appears to prevent the Ab-independent activation of complement (C). The CP also blocks the access of Abs to noncapsular antigens such as the lipopolysaccharide and most (but not all) outer membrane proteins. Sensitization of host erythrocytes. The CP is released during systemic infection and can circulate long after bacterial clearance. The CP can sensitize red cells to hemolysis by Ab and C, and may contribute to the anemia frequently found in Hib sepsis. Immunogenicity. Circulating Abs to the CP mediate phagocytosis and bacteriolysis, and secretory Abs may also contribute to immunity. Both the primary and (some undefined) secondary structure of the CP appear to be recognized by Ab. The human anti-CP response to Hib infection or CP vaccine is delayed in maturation. CP coupled to protein carriers can elicit mature-type Ab responses in infants and also prime an early-for-age responsiveness to uncoupled CP.

H 018 LIPOOLIGOSACCHARIDE AND VIRULENCE OF HAEMOPHILUS INFLUENZAE, Eric J. Hansen, Department of Microbiology, University of Texas Health Science Center, Dallas TX 75235.

The lipooligosaccharide of Haemophilus influenzae type b has been shown to play a role, secondary to the type b polysaccharide capsule, in the expression of virulence by this pathogen. Animal passage of a relatively avirulent strain of H. influenzae type b resulted in the selection of a fully virulent variant of the original parent strain. Sodium dodecyl sulfate-polyacrylamide gradient gel electrophoretic analysis determined that the fully virulent strain synthesized a different lipooligosaccharide molecule than the avirulent parent strain. Western blot analysis with monoclonal antibodies revealed that the lipooligosaccharide of the fully virulent variant had an oligosaccharide region different from that of the avirulent parent strain. Colony blot analysis with these same monoclonal antibodies showed that virulent variants of the avirulent strain arose spontaneously in vitro by a two-step process at frequencies ranging from 0.3 - 1.2%. The fully virulent variants were also shown to be resistant to killing by normal serum from infant rats, while the avirulent strain was exquisitely sensitive to normal serum bactericidal activity. Repeated single colony passage of thirty strains of H. influenzae type b showed that most strains had stable expression of lipooligosaccharide surface epitopes. However, when 500-2000 colonies of each strain were analyzed in a colony blot-RIA, every strain studied in this manner was found to exhibit antigenic variation in lipooligosaccharide expression. These changes in the antigenic characteristics of the lipooligosaccharide were sometimes associated with changes in both the relative level of virulence of the strain and resistance to the bactericidal activity of normal serum. These data indicate that lipooligosaccharide phenotypes are not stably expressed by H. influenzae type b strains growing in vitro.

H 019 CAPSULAR POLYSACCHARIDE OF TYPE III GROUP B STREPTOCOCCUS, Dennis L. Kasper, Michael R. Wessels, Craig E. Rubens, Harold J. Jennings, Channing Laboratory, Harvard Medical School, Boston, MA, University of Washington, Seattle, WA, and National Research Council of Canada, Ottawa, Ont.

The type III group B streptococcal capsular polysaccharide is an important surface structure in virulence and immunity to these organisms. Protective antibodies are primarily directed against this structure which is composed of galactose, glucose, N-acetyl glucosamine and sialic acid existing in a 2:1:1:1 molar ratio. A structure has been proposed for this polysaccharide antigen consisting of a trisaccharide backbone repeating unit with a disaccharide side chain. Each pentasaccharide repeating unit has a terminal side chain sialic acid residue which appears to exert conformational control over the immunodeterminant.

We have derived oligosaccharides from the capsular polysaccharide of type III group B Streptococcus by enzymatic hydrolysis of a specific backbone glycosidic bond utilizing an endo-β-galactosidase from Flavobacterium keratolyticus. Enzymatic digestion of the polysaccharide produces oligosaccharide fragments of one or more pentasaccharide repeating units. Structural analysis of the pentasaccharide indicated that the terminal side chain sialic acid residue of the polysaccharide is linked to O-3 of the penultimate galactopyranosyl residue, rather than to O-6, as had been proposed previously. Thin layer chromatogram binding assay and radioactive antigen binding assays with radiolabeled oligosaccharides demonstrated the single repeating unit pentasaccharide oligosaccharide to be poorly antigenic. Increasing oligosaccharide size to a decasaccharide consisting of two repeating units resulted in an 8 fold increase in antigen binding in direct radioactive antigen binding assay. The results suggest that a region of the immunodeterminant site critical for antibody binding is located in the backbone of the polysaccharide.

Indirect evidence has suggested the capsular polysaccharide of group B Streptococcus (GBS) is an important factor in bacterial virulence. To study directly the role of the capsule, we have derived an unencapsulated mutant from a (wild type) type III GBS strain by transposon mutagenesis. Tn916, a 10Kd transposon, was transferred by conjugation from Streptococcus faecalis. GBS transconjugants were screened for expression of capsular polysaccharide by an immunoblot assay. One of eleven non-reactive transconjugants (COH31-15) was further characterized: an extract of COH31-15 failed to inhibit a competitive ELISA for type III polysaccharide, and immune electron microscopy showed no extracellular capsule. Southern blot of a restriction endonuclease digest of the DNA from COH31-15 showed a single insertion site of the transposon. Preliminary data in a neonatal rat model of GBS infection indicate at least a 2 log increase in LD_{50} for COH31-15 over the wild type percent GBS strain.

H 020 DEACYLATION OF BACTERIAL LIPOPOLYSACCHARIDES BY NEUTROPHILS. Robert S. Munford, University of Texas Health Science Center, Dallas, TX 75235.

The molecular basis for the stimulation of animal cells by gram-negative bacterial lipopolysaccharides (LPS) is not known. Similarly, little is known about host mechanisms for inactivating or detoxifying LPS. Understanding how animal cells catabolize LPS should help clarify these issues.

The lipid A region of LPS is a glucosamine disaccharide that has covalently linked phosphates, acyl chains, and a polysaccharide chain. In lipid A of enterobacterial LPS, four molecules of 3-hydroxytetradecanoate (3-OH-14:0) are linked directly to the glucosamine backbone, and normal fatty acids (dodecanoate, tetradecanoate) are substituted to the hydroxyl groups of some of these 3-OH-14:0 residues to form acyloxyacyl linkages. We found that human peripheral blood neutrophils have an enzymatic activity that breaks the acyloxyacyl linkages, releasing the normal fatty acids (acyloxyacyl hydrolysis).

We have now partially purified acyloxyacyl hydrolase from human promyelocytes (HL-60 cells) and studied the impact of enzymatic deacylation on the bioactivities of LPS. Although the studies are incomplete, three findings are noteworthy. First, enzymatic deacylation decreases the potency of LPS in some assays (murine spleen cell mitogenicity, rabbit pyrogenicity test) by a factor of only 10 to 20. Second, in other assays (dermal Shwartzman reaction, stimulation of human umbilical vein endothelial cells to express neutrophil adhesion molecule[s]), enzymatically deacylated LPS are 100- to 1,000-fold less potent than control LPS. Third, enzymatically deacylated LPS block the stimulation of human umbilical vein endothelial cells by LPS (T. Pohlman and J. Harlan, University of Washington, Seattle); several characteristics of the inhibition suggest competition between deacylated and acylated LPS for a common (?receptor) site on the endothelial cells.

The effects of enzymatic deacylation on the bioactivities of LPS are thus striking, yet the biological role of acyloxyacyl hydrolysis is poorly understood at this time. One hypothesis is that neutrophil acyloxyacyl hydrolase deacylates the LPS in ingested bacteria. Deacylated LPS, released from the neutrophils by exocytosis or cell death, would have greatly reduced tissue toxicity yet might interact with other cells to (1) stimulate nontoxic components of the inflammatory response, and/or (2) block stimulation by acylated LPS. It should be noted, however, that we have used purified LPS for these studies, not the LPS in intact bacteria; that exocytosis of deacylated LPS by neutrophils has not been shown; and that the basis for the ability of deacylated LPS to block LPS stimulation is not known. Much more work is needed to determine the function(s) of acyloxyacyl hydrolases in neutrophils and other animal cells.

H 021 STRUCTURE AND IMMUNOCHEMISTRY OF STAPHLOCOCCUS AUREUS CAPSULAR POLYSACCHARIDES, Willie F. Vann, Ann Sutton, Monique Moreau, and W.W. Karakawa, Office of Biologics Research and Review, 8800 Rockville Pike, Bethesda, MD. 20892 and Department of Microbiology and Biochemistry, Pennsylvania State University, University Park, PA.

S. aureus is a major cause of nosocomial infections. Control of the organism is becoming increasingly difficult due to antibiotic resistance. The primary means for epidemiological characterization of S. aureus are phage typing and antibiotic resistance patterns. Karakawa et al. have demonstrated the presence of antiphagocytic capsular polysaccharides on the surface of clinical isolates of S. aureus (1). Using specific polyclonal and monoclonal sera, 11 serologically distinct types have been demonstrated. The most prevalent in human disease are types 5 and 8. Antisera against these two types show specific opsonization.

The polysaccharides are cell bound and are structurally similar to teichuronic acids. The structure of the type 5 and 8 polysaccharides has been determined by specific degradation, methylation analysis, and NMR methods. Both polysaccharides are composed of N-acetylfucosamine and N-acetylmannosaminouronic acid.

Type 5 $\rightarrow 4)manNAc A\beta(1\rightarrow 4)fucNAc\beta(1\rightarrow 3)fucNAc\beta(1\rightarrow$

Type 8 $\rightarrow 3)(4\text{-}OAc)manNAc A\beta(1\rightarrow 3)fucNAc\alpha(1\rightarrow 3)fucNAc\beta(1\rightarrow$

Types 4 and 5 differ only in the location of O-acetyl groups. Type 8 is both structurally and serologically identical to the polysaccharide of the T strain reported by Wu and Parks (2).

The nature of shared determinants and of cross-reactions between staphylococcal and chemically similar polysaccharides have been investigated by ELISA. The O-acetyl group is an important serological determinant for the type 8 polysaccharide. The carboxyl group is an important part of the core structure for both type 5 and 8. The cross reactivity of type 8 and pneumococcal type 4 polysaccharides is associated with a common structure containing a 4-substituted N-acetylmannosamine. The cross-reactions between S. aureus types 4 and 5 polysaccharides and the pneumococcal type 12 polysaccharide is associated with a common trisaccharide sequence.

1. Karakawa, W.W. and D.A. Young. Infect. Immun. 25:175 (1979)
2. Wu, T.C.M. and J.T. Park. J. Bacteriol. 108:874 (1971)

Entry of Pathogens into Host Cells or Tissues

H 022 **GENETIC, BIOCHEMICAL, AND IMMUNOLOGICAL STUDIES OF THE ENTEROINVASIVE PHENOTYPE IN SHIGELLA SPP.,** **Thomas Larry Hale and Samuel B. Formal, Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, D.C. 20307.**

The process of mammalian cell invasion by Shigella spp. and enteroinvasive strains of Escherichia coli has been studied in the HeLa cell model. Experiments using HeLa cells prelabeled with H^3-uridine indicate that invasion does not induce release of labeled mRNA, and pretreatment of HeLa cells with cytochalasin B, iodoacetate, or 2,4-dinitrophenol inhibits the uptake of shigellae. These data suggest that bacterial invasion is an energy requiring process which involves microfilament polymerization and leaves the plasma membrane intact. Transmission electronmicroscopy of HeLa cells exposed to virulent shigellae indicates that invasion is an induced endocytic event which is mediated by sequential of binding of bacterial surface ligands to plasma membrane receptors. Proteolytic digestion of shigellae negates the invasive phenotype, so the bacterial ligands inducing endocytosis are apparently outer membrane protein(s). Genetic analysis has shown that the invasive phenotype is associated with the expression of a complement of seven polypeptides encoded by 140 or 120 MDal plasmids in Shigella spp. and E. coli. Two of these polypeptides, which have been designated "b" (57 KDal) and "c" (43 KDal), are exposed on the cell surface, and these outer membrane proteins have a proclivity for binding to the HeLa cell surface. These data suggest that polypeptides b and c may be involved in receptor-mediated uptake of shigellae. Analysis of the immune response to shigella infection in both Rhesus monkeys and humans has revealed that five plasmid-coded polypeptides are recognized by serum and mucosal antibody in convalescence. In addition to polypeptides b and c, these antigens include a 140 KDal species and polypeptides designated "a" (78 KDal) and "d" (35 KDal). Antibody evoked by S. flexneri 2a infections cross reacts with the plasmid-coded polypeptides of all other Shigella species, but the strongest protective immunnity is serotype specific.

H 023 PARASITE-DIRECTED ENDOCYTOSIS, Zell A. McGee, Center for Infectious Diseases, Diagnostic Microbiology and Immunology, University of Utah School of Medicine, Salt Lake City, UT 84132.

Gonococci appear to induce their own endocytosis and transport across human fallopian tube epithelium by low columnar cells. We have designated these cells "Trojan horse cells" because they transport an enemy across the normally protective epithelial barrier. Similar cells elsewhere in the body transport other invasive bacterial and sometimes viral pathogens across an otherwise protective epithelial barrier. The process of endocytosis is generally presaged by a vigorous elongation of microvilli which appear to seek out bacteria attached elsewhere on the cell surface and to entrap the bacteria against the surface prior to formation of endocytic vacuoles, which are then transported from the luminal to the basilar pole of the cell. After some time at the basilar pole of the cell, the vacuole develops channels opening from the vacuole into the subepithelial tissues; the bacteria pass through these channels and exit into the subepithelial tissues by a process of exocytosis which appears to be the reverse of the endocytosis that took place at the luminal pole of the cell. The mechanisms by which microorganisms such as gonococci, meningococci and chlamydia induce their own endocytosis are unknown, but parasite-directed endocytosis appears to be an important element in disease caused by a wide variety of microorganisms pathogenic for humans.

H 024 IDENTIFICATION OF THE LIGANO THAT ALLOWS YERSINIA TO ENTER ANIMAL CELLS, Ralph R. Isberg, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111

The ability to invade and survive within host cells is a property of many pathogenic bacteria. We are studying Yersinia pseudotuberculosis as a model pathogen in order to gain detailed information on the mechanism of entrance into an epithelial cell. We have identified the genetic locus encoded by Y. psendotuberculosis that allows it to enter cultured mammalian cells by selecting for E. coli strains, harboring Yersinia DNA, that have the same invasive phenotype. Clones identified in this manner harbor a single Y. pseudotuberculosis gene, called inv. Mutations in inv, constructed in E. coli render Y. pseudotuberculosis unable to enter animal cells.

Genetic and biochemical analyses show that inv encodes a single polypeptide, 108 kDal in length. The protein is localized on the bacterial cell surface. By purifying this protein, and by developing cell binding assays to solid matrices, we have shown that this 108 kDal species, called invasin, binds the bacteria to the animal cells as the initial step in the entry process.

Genetics of Bacteria-Host Cell Interaction

H 025 ANTIGENIC VARIATION IN BORRELIA, Alan G. Barbour, Departments of Microbiology and Medicine, University of Texas Health Science Center, San Antonio, TX 78284.

The relapsing fever Borrelia spp. undergo an antigenic variation in their hosts that is as extensive as that found in infections with the African trypanosomes. The serotypic determinants for one strain of Borrelia hermsii are abundant surface proteins, the VMP's. The VMP's differ from one another in their molecular weights, antibody reactivities, and primary structures. The genes encoding the VMP's can be found in two different DNA environments: "silent" and "active". In serotype 7, for example, a sequence specifying the VMP7 protein is present in both the silent and active environments. In these same serotype 7 cells, however, a sequence encoding the serotype-specific protein for serotype 21 can only be found in the silent environment. An antigenic switch from serotype 7 to serotype 21 is the result of replacement of the vmp7 gene in the active locus with a copy of a vmp21 gene from a silent locus; this recombinational event appears to be non-reciprocal. Further study of the organization of genetic material in B. hermsii showed that these organisms have linear plasmids. The silent forms of the vmp genes are on one group of linear plasmids and the active or expression-linked copy of the gene is on another plasmid. In a variant that expresses a novel VMP protein and that is incapable of switching to other serotypes several kilobases of sequence which defines the active locus had translocated to a third variety of linear plasmid unique to these non-switching variants.

H 026 MUTANTS OF *SALMONELLA TYPHIMURIUM* THAT CANNOT SURVIVE WITHIN MACROPHAGES ARE AVIRULENT. Patricia I. Fields, Ronald V. Swanson, and Fred Heffron, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA.

Salmonella typhimurium is an intracellular pathogen of mice and a major cause of gastroenteritis in man. We have developed a simple in *vitro* assay to identify mutants that are attenuated for survival within macrophages. Using this assay, we identified 82 Tn*10* insertion mutations in *S. typhimurium* (designated MS mutations) amoung 9516 tested that confer a diminished capacity to survive within mouse macrophages in vitro. All the MS mutants are also less virulent in vivo, indicating that survival in the macrophage is essential for virulence in this pathogen. Also, all the MS mutations are identical to the parent for several growth characteristics. Thus, the mutants contain defects that affect survival within macrophages, but are normal in other respects. Phenotypic characterization of the MS mutants has identified defects for 43: 12 are auxotrophs, 9 are altered in their response to oxidants, 6 are hypersensitive to serum, 1 Tn*10* insertion is in the plasmid associated with virulence in this organism, 8 are nonmotile, 1 possesses an LPS defect.

We believe that many of the mutants may represent mutations in previously unidentified mechanisms for intracellular survival. Further characterization of the MS mutants should elucidate the mechanisms employed by *S. typhimurium* for intracellular survival and may be applied to the study of other clinically important but more complex intracellular pathogens.

Most of the MS mutants have been tested for their ability to protect a mouse from challenge with the virulent parent strain. A wide range of protective properties was seen for the mutants tested, from completely protective to completely nonprotective. Most of the mutants were protective, suggesting that this method of isolating mutants may be applicable to the isolation of live attenuated vaccine strains in this and other intracellular pathogens.

H 027 CHARACTERIZATION OF A FAMILY OF PLASMIDS MEDIATING ESSENTIAL VIRULENCE TRAITS IN SALMONELLA SEROTYPES. Donald Guiney, Joshua Fierer, Gary Chikami, and Paul Beninger. University of California, San Diego, School of Medicine, San Diego, CA 92103

Plasmids of 30-60 M dal (45-90 kb) in size have been implicated in the virulence of several non-typhoid Salmonella serotypes. Salmonella dublin is host adapted to cattle and is shed from carriers in the milk, sometimes causing fatal septicemias in calves. S. dublin strains are particularly invasive in humans, and the disease can be paralleled by oral inoculation of BALB/c mice. Virulent S. dublin strains in mice are able to invade through the bowel and cause fatal systemic disease. The virulent, wild-type S. dublin Lane strain contains an 80 kb plasmid, pSDL2, which we have shown previously is essential for the virulence of S. dublin Lane in mice. We used the Tn5-oriT transposon (a derivative of Tn5 containing the transfer origin region of RK2) to label pSDL2 with kanamycin resistance and to facilitate transfer of pSDL2 between strains using the IncP conjugation system of RK2. A plasmid-free derivative of S. dublin Lane, designated LD842, is avirulent for mice and re-introduction of pSDL2 into LD842 restored virulence. A study of the natural history of oral infection in mice, comparing S. dublin Lane containing pSDL2 with the cured strain LD842, has shown that the virulence plasmid is not required for initiation of the infection in the gut, nor spread to regional lymphatics or the reticuloendothelial system, but is essential to establish a progressive systemic infection. A restriction map of pSDL2 has been constructed and regions encoding essential replication and virulence functions have been located. Use of Tn5-oriT mutagenesis has localized a region required for virulence within a 6.4 kb portion of the Sal I B fragment. Several EcoRl generated deletions have been constructed which eliminate up to 60% of the plasmid but still retain the replication and virulence phenotypes. LD842 containing an avirulent Tn5-oriT insertion in pSDL2 is an effective live, oral vaccine in mice. Following spontaneous clearance of the vaccine strain, mice are protected from challenge with the virulent S. dublin Lane strain. Plasmids with markedly different restriction patterns were found in an S. dublin strain expressing Vi antigen (pSDVl) and an S. enteriditis strain (pSEl). Both pSDVl and pSEl were labeled with the Tn5-oriT transposon and transferred into the plasmid-free S. dublin LD842. Both plasmids fully restored the virulence of LD842 for mice, indicating that despite substantial differences in restriction patterns, the virulence regions of these plasmids have been conserved, and that the virulence plasmid from one serotype (enteriditis) can function in a different serotype (dublin). These results suggest that a family of related plasmids in Salmonella mediate similar virulence traits that are essential for systemic infection.

H 028 GENETICS OF PILUS EXPRESSION AND FUNCTION. M. So*, H.S. Seifert*, C.D. Deal*, N. Stromberg+, K.A. Karlsson+, G. Nyberg#, S. Normark#. *Dept. of Molecular Biology Scripps Clinic and Research Foundation, La Jolla, Ca. U.S.A. +Dept. of Medical Biochemistry, Univ. of Goteborg, Goteborg Sweden. #Dept. of Microbiology, Univ. of Umea, Umea, Sweden.

Pilin expression in N. gonorrhoeae is controlled at two expression loci pilE1 and pilE2. These two chromosomal loci contain complete pilin structural gene and promoter sequences while several other loci contain only truncated variant pilin sequences. Although both pilE1 and pilE2 can be used, only one is needed for full pil expression (1). pilE2 in gonococcal strain MS11 was marked with the "CAT" cartridge under pil promoter control. This strain still has an intact wild type pilE1, and is therefore pil+ (P+) and CmR. We have used this P+,CmR strain to show that gene conversion can occur between the two expression sites, and between a pilE locus and a major silent pil locus, pilS1.

Pilus phase variation can be controlled by three mechanisms. Single base mutations in the pil gene results in premature translation stops, assembly defective pilins, and abolition of transcription (2). P- variants can also result from a deletion of the pil gene and promoter sequences from the expression sites (1). We have recently identified a third regulatory mechanism involving trans acting factors which act on pilin promoter sequences. Data will be presented which show that these trans acting elements are related to nifA and ntrA which regulate nitrogen fixation and nitrogen utilization genes.

As part of an on going program to examine gonococcal pilus biology, we have identified (in collaboration with K.A. Karlsson's lab and S. Normark's lab in Sweden) glycolipid receptors on epithelial cells recognized and bound by the bacterium. Using purified receptor as probe, we have identified a protein adhesin which is involved in the attachment process. This adhesin is immunologically and biochemically distinct from pilin, but which appears to be associated with the major pilin subunit. Details of these binding studies will be discussed.

1. Segal et al., Cell 40:293-300 (1985)
2. Bergstrom et al., PNAS 83:3890-3894 (1986)

H 029 PHASE AND ANTIGENIC VARIATIONS OF GONOCOCCAL PILIATION INVOLVE PILIN GENE CONVERSION, John Swanson[1], J. Michael Koomey[2], and John Boslego[3], Lab of Microbial Structure & Function, NIAID, NIH, RML, Hamilton, MT 59840[1], Lab of Bacteriology, The Rockefeller University, NYC, NY 10021[2], and Div. of Bacterial Diseases, WRAIR, WRAMC, Washington, DC 20307[3].

Gonococci (Gc) change both their piliation phase ($pilus^+ \rightleftarrows pilus^-$) and the structure/antigenicity of their pilin/pilus ($pilin_a^+ \rightarrow pilin_b^+$) through recombinationally-substituting nucleotide sequence of a stored, partial pilin gene for the analogous stretch in their single expressed, complete pilin gene. Most such resulting new chimeric complete pilin gene encodes an "orthodox" pilin whose synthesis correlates with pilus formation and $pilus^+$ phenotype; but expression of pilS1 copy 5 partial gene's sequence in such a chimeric pilin gene results in pilin synthesis but $pilus^-$ status (P^-rp^+ phenotype).

Gc recovered from two experimentally-infected males express structurally/antigenically-distinct pilins compared with "input" Gc. Differences observed among these in vivo variants of strain $MS11_{mk}$ derive from gene conversions not only by different partial pilin genes but also by different length-stretches of the same partial gene; analogous phenomena are also seen among the strain's in vitro variants. A well-characterized partial gene, pilS1 copy 2, was expressed by one infected male's in vivo variants. Both in vivo and in vitro variants express pilin polypeptides containing changed amino acid sequences; these changes occur mainly in the pilin molecules' so-called "semi-variable" domains, but changes also appear in the pilins' "hyper-variable" and "conserved" domains.

The observed changes in pilin gene and polypeptide sequences document an ability of Gc to express any of a large variety of pilin subunits; the magnitude of such pilin variation rests on the Gc genome's repertoire of multiple partial pilin genes that a) are largely homologous but encode distinct pilin antigens and b) can donate variable length stretches of sequence for recombinational creation of novel chimeric complete pilin genes.

Immunology, Membrane Proteins, LPS and Capsules

H 100 FATTY ACYLATED INTEGRAL SURFACE MEMBRANE PROTEINS OF MYCOPLASMAS ARE ABUNDANT AND EXPRESS MARKED INTRASPECIES VARIATION. Michael J. Boyer and Kim S. Wise. University of Missouri, Columbia, MO 65212.
Mycoplasma surface proteins mediate interactions with host cells and contribute to antigenic variation within species. Two monoclonal antibodies (mAb) were used to define distinct sets of hydrophobic integral membrane surface proteins of Mycoplasma hyorhinis, each exhibiting marked size variation among six isolates of this species. Metabolic labeling of isolates with ^{3}H-palmitate and subsequent analysis by Triton X-114 phase fractionation and SDS-PAGE demonstrated that virtually all of approximately 25 palmitate-labeled proteins were hydrophobic and many were strikingly polymorphic. Radioimmunoprecipitation with mAbs to surface proteins p23 and p120 (defined on one prototype strain of M. hyorhinis) showed that variant antigens on all strains were ^{3}H-palmitate labeled integral proteins. To determine the extent and type of FA linkage among membrane proteins of M. hyorhinis, a prototype strain was labeled with ^{3}H-palmitate, TX-114 fractionated membrane proteins were separated by SDS-PAGE, and gels were treated with alkaline hydroxylamine. While most proteins were resistant to this treatment (suggesting probable amide linkage), the variant protein p120 showed loss of FA (suggesting thioester or O-ester linkage). To determine the actual lipid species linked to proteins in ^{3}H-palmitate-labeled cells, protein-bound lipids were released by acid methanolysis and products were analyzed by HPLC. Most (87%) of the label was recovered as palmitate methyl ester and a small percentage as palmitate. No other FA derivatives were observed. This provided direct evidence that palmitic acid was covalently attached to abundant and variable integral membrane surface proteins of M. hyorhinis.

H 101 Effect of Loss of Escherichia coli (RDEC-1) Adherence Pili on Diarrheal Disease in the Rabbit. J. R. CANTEY*, L. R. INMAN, R. K. BLAKE, VA Med. Ctr. and Med. Univ. of SC, Charleston, SC.
E. coli strain RDEC-1 is an enteroadherent bacterium that adheres to Peyer's patch and gut mucosa in the rabbit. AF/R1 pili mediate adherence to Peyer's patch M cells in vivo and ileal mucosal brush borders in vitro. Dr. Samuel Formal prepared a non-piliated RDEC-1 strain (42-2-37-8). Compared to the parent RDEC-1 strain (35 rabbits) the 42-2-37-8 strain (41 rabbits) took longer to colonize the gut (3 versus 1.9 days, $p<0.001$), failed to colonize 4 of 41 rabbits ($p=0.016$), caused less diarrhea (37% versus 71%, $p=0.001$) and fewer deaths (12% versus 43%, $p=0.0126$). Light microscopic studies in 18 rabbits revealed that both strains adhered to absorptive epithelium but the 42-2-37-8 adhered less frequently to Peyer's patches (10% versus 75%, $p<0.001$). The electronmicroscopic appear-ance of the adherence of the 42-2-37-8 strain did not differ from the parent strain. AF/R1 pili are not necessary for adherence but increase the virulence of the RDEC-1 strain.

H 102 A NEW MODE OF VIRULENCE: THE C5a STREPTOCOCCAL PEPTIDASE, P. Cleary and S. O'Connor, University of Minnesota, Minneapolis, MN 55455.
Virulent S. pyogenes has long been known to resist uptake by human phagocytic cells. Recently, however, we have discovered another mechanism of avoiding the phagocytic response, these organisms circumvent immune detection by eliminating the primary chemotactic signal, C5a. This streptococcal inhibitor (SCP) has been shown to cleave C5a at lys68, producing a peptide which is unable to bind to C5a receptors on PMNs. To investigate that the role SCP might play in pathogenesis, SPC^{-} mutants have been isolated by Tn916 insertional and chemical mutagenesis for comparison to wild type SCP^{+} cells in animal models. Injection of SCP^{+} and SCP^{-} isogenic cells into the mouse peritoneum has shown that SCP retards the inflammatory response by delaying the accumulation of PMNs at the site of infection. Antibody known to neutralize SCP activity in vitro, also eliminates it's activity in vivo and stimulates the influx of PMNs into the peritoneum. These results and the discovery of this activity in other species suggests that chemotaxis inhibition is a general mechanism of pathogenesis.

H 103 IDENTIFICATION OF *TREPONEMA PALLIDUM* OUTER ENVELOPE COMPONENTS BY TRITON X-114 (TX-114) PHASE PARTITION, Thomas M. Cunningham, James N. Miller, and Michael A. Lovett. UCLA, Los Angeles, CA 90024.

Several potential *T. pallidum* outer envelope proteins were identified after removal of *T. pallidum* surface components by TX-114 detergent treatment. Polypeptides solubilized from intact, percoll purified organisms partitioned into hydrophobic (warm TX-114) or hydrophilic phases. Proteins that partitioned into the hydrophobic phase may have done so by virtue of hydrophobic anchor or transmembrane domains. Antigenic polypeptides of apparent molecular weights 47, 38, 34, 29, 20, 16, 14 kd were identified in the hydrophobic phase by SDS-PAGE and Western blotting against human secondary syphilitic sera. The well-characterized 4D antigen was also seen in TX-114 soluble extracts. No flagellar components were detected by Western blotting against high titer anti-flagellar sera in TX-114 solubilized material. In addition, recently identified *T. pallidum* penicillin binding proteins did not solubilize in TX-114. These data, together with dark-field and electron microscopic examinations, suggest that the inner membrane of *T. pallidum* was left intact by TX-114 treatment.

H 104 IN VIVO TREATMENT OF BALB/c MICE WITH ANTI-L3T4 (GK1.5) ANTIBODY REDUCES PFC RESPONSES TO E. COLI LPS, Karen L. Elkins, R. Mark Buller, Philip W. Stashak, and Phillip J. Baker, NIH, Bethesda, MD 20892.

Recently we have shown that low dose priming of BALB/c mice with E. coli 0113 lipopolysaccharide (LPS) increases direct (IgM) plaque forming cell (dPFC) responses when mice are subsequently challenged with optimal doses of E. coli 0113 LPS, while priming with E. coli 055 LPS or with S. marcescens LPS decreases (suppresses) the dPFC response upon homologous challenge. Here we further investigate the mechanism of development of suppression of dPFC responses. Although low dose priming generated no detectable dPFC, small amounts of serum antibody were found using a LPS specific ELISA. However, the presence of these small amounts of antibody could not be correlated with the eventual development of suppression. Further, passive transfer of small quantities of LPS specific antibody had no effect on the dPFC response, although transfer of large amounts did suppress the dPFC response slightly. Therefore the appearance of small quantities of serum antibody following low dose priming does not appear to be responsible for the observed suppression of the dPFC response. However, depletion of $L3T4^+$ cells in vivo in either BALB/c or C57Bl/6J (by treatment with GK1.5 antibody), followed by immunization with E. coli 055 LPS, significantly reduces the magnitude of the LPS specific dPFC primary response compared to untreated mice. These results suggest that both $L3T4^+$ and $L3T4^-$ cells compete in the regulation of specific dPFC responses to E. coli 055 LPS, and may play a role in the development of suppression following LPS low dose priming.

H 105 PREVENTION OF PERITONITIS BY A LYMPHOKINE/MONOKINE, J. Gordon, M. MacPhee, I. Zakaluzny, J. Marshall, J.C. Puyana, J.L. Meakins, N.V. Christou and H. Rode, McGill University/Royal Victoria Hospital, Montreal, Canada.

Anergic patients (those who fail to give a DTH response to recall antigens) have a 10-fold greater risk of postoperative mortality related to peritonitis as compared to reactive individuals. To study this phenomenon we developed a rat model of anergy consisting of a 30% third degree thermal injury: such animals had an increased mortality when injected i.p. with a mixture of enteric bacteria and $BaSO_4$. We report here that these rats could be protected by the coinjection with the bacteria of a supernatant derived from cultures of human leukocytes activated in MLC. Control animals injected with saline, with supernatant from non-activated cells or with zymosan activated serum (to attract PMN) died with disseminated infections; those injected with MLC supernatants and survived showed no infections. The MLC supernatants, free of antibodies, may have been effective by mobilizing and/or activating mononuclear cells.

Supported by the Medical Research Council of Canada.

H 106 DEVELOPMENT OF IgM ANTIBODY TO GROUP B STREPTOCOCCUS TYPE III (GBS) IN HUMAN INFANTS. S.P. Gotoff, C.K. Papierniak, M.E. Klegerman, M.E. Rauen, K.M. Boyer, Department of Pediatrics, Rush-Presbyterian-St.Luke's Medical Center, Chicago, IL

We developed a direct quantitative ELISA specific for human serum IgM antibody to the polysaccharide antigen of GBS III similar to our IgG ELISA (JID 153:511, 1986) which was standardized by "double-label" solid phase immunoassay. The mean IgM anti-GBS III in 94 adults was 5 ug/ml while 12 cord sera contained <.02 ug/ml. Longitudinal studies in 20 infants during the first year of life revealed acquisition of levels $\geq$ 0.3 ug/ml in all infants with a mean value >1.0 ug/ml at a year of age.

Sera containing IgM anti-GBS III administered intraperitoneally protected 2-3 day old Sprague-Dawley rats against a subcutaneous LD_{90} challenge with GBS III strain M732. Subgroups of neonatal rats were bled at the time of challenge to determine levels of IgM and IgG anti-GBS III after passive immunization with serial dilutions of sera containing either IgM or IgG anti-GBS III. Levels of 0.1 ug/ml of IgG anti-GBS III in the rats afforded full protection. Significant protection was observed in rats who had IgM anti-GBS III levels $\geq$0.3 ug/ml. Sera containing low levels of IgG and IgM anti-GBS III were not protective, including sera from patients with macroglobulinemia. Thus, development of IgM specific antibody to GBS III may contribute to the age-limited incidence pattern of neonatal GBS disease.

H 107 REQUIREMENTS FOR THE EXPRESSION OF THE MICROBIOSTATIC ACTION OF MACROPHAGES. D. L. Granger, J. R. Perfect and D. T. Durack, Duke University, Durham, N. C. 27710.

Relatively little attention has been paid to the microbiostatic capability of phagocytes. Yet for certain facultative intracellular parasites, acquired resistance is expressed primarily as a "restraining power" within tissues leading to quiescent infection and long-term survival of the host. Reanimation of arrested organisms following immunosuppression shows that the host-parasite relationship is a dynamic interaction in which both parties are poised against one another.

Macrophages function as essential effector cells for arresting microbial replication. We have studied this process in vitro using murine peritoneal macrophages and the pathogenic fungus, Cryptococcus neoformans. It is well recognized that lymphokine-induced macrophage activation usually, but not always, followed by exposure to a microbial product is necessary for full antimicrobial function. Given these conditions, are there additional requirements for expression of macrophage fungistatic action? We have found at least two: (1) macrophages must have access to a macromolecule found in normal serum. This is a protein which is not a lymphokine or an opsonin; (2) a small molecule present in ordinary cell culture medium must be present. Without either of these factors in their environment, activated macrophages cannot express fungistatic capability. Our poster will present data on purification and identification of these two factors. The results bear upon the mechanism of macrophage microbiostasis. Furthermore, this knowledge may give insight into understanding reactivation of dormant infections.

H 108 IMMUNOGENETICS AND IMMUNE RESPONSE: IMMUNOCHEMICAL ANALYSIS OF IMMUNE RESPONSE TO CHLAMYDIA TRACHOMATIS IN REITER'S SYNDROME AND NON-SPECIFIC URETHRITIS, R.D. Inman, M.E.A. Johnston, B. Chiu, J. Falk, M. Petric.

Chlamydia trachomatis (Ct) has been proposed as a causative agent in Reiter's syndrome (RS) when such an infection occurs in a host who is HLA B27-positive, the susceptibility marker for the disease. To assess whether this susceptibility is reflected in a characteristic humoral immune response we compared patients with complicated (RS) and uncomplicated courses of nonspecific urethritis (NSU). Geometric mean titers of antibodies to C. trachomatis by immunofluorescence were 89.6 for RS, 80.0 for NSU and 16.0 for normals. ^{125}I Protein A probing of immunoblotted antigens of C. trachomatis revealed no band unique to RS, however reactivity with the 36,500 dalton antigen was present in 5/6 RS and 1/6 NSU. ^{125}I-anti-IgA probing of immunoblots demonstrated reactivity with the 59,000 dalton antigen in 6/6 RS and 2/6 NSU. The major outer membrane protein of C. trachomatis (40,000 daltons) bound immunoglobulin nonspecifically. There was no clear differentiating feature between HLA-B27-positive and B27-negative RS. One sequentially studied patient revealed an augmentation in synovial fluid IgA reactivity during the course of disease. No pattern of humoral immune response to C. trachomatis could be regarded as specific for RS nor for HLA B27-positivity. This study although not identifying a RS-specific antigen in Ct demonstrates the usefulness of Western blotting in defining immunogenetic aspects of host immune response to microbial antigens.

H 109 THE ROLE OF CAPSULE IN THE VIRULENCE OF CYTOTOXIC HAEMOPHILUS PLEUROPNEUMONIAE, Thomas J. Inzana and Ma Jianneng, Washington State University, Pullman, WA 99164. Haemophilus pleuropneumoniae is an encapsulated, cytotoxic, Gram-negative bacterium that causes necrotizing, hemorrhagic pneumonia in swine. The role of the capsule in bacterial virulence, and antibody to the capsule in host protection, was investigated. Antibody to whole cells and monospecific or monoclonal antibody to capsule was not bactericidal for encapsulated H. pleuropneumoniae. However, a capsule-deficient mutant was sensitive to killing by 10% normal swine serum in the presence or absence of EGTA-Mg^{++}. Encapsulated H. pleuropneumoniae incubated with whole cell antiserum, or monospecific or monoclonal antibody to the capsule, were sensitive to phagocytosis by swine neutrophils. Phagocytosis did not occur if the bacteria were incubated with normal rabbit or swine serum. In contrast, a capsule-deficient mutant incubated in normal rabbit or swine serum was efficiently taken up by swine neutrophils. Mice immunized with purified lipopolysaccharide (LPS) or to capsule covalently conjugated to bovine serum albumin raised ELISA antibody titers of 1:320 or greater to the immunizing antigen, but the mice were not protected against lethal, intranasal challenge. Mice immunized with live, whole cells, however, had ELISA titers of 1:160 or greater to capsule, LPS, and non-LPS somatic antigens, and were 100% protected against disease. Mice immunized with a capsule-deficient mutant had 1:640 or greater ELISA titers to somatic antigens, but less than 1:10 to the capsule, and were also protected against lethal disease. Thus, the capsule of H. pleuropneumoniae inhibited phagocytosis and the bactericidal activity of antiserum, but in contrast to studies with noncytotoxic encapsulated bacteria, antibody to the capsule was not protective.

H 110 IMMUNITY TO INTRACELLULAR BACTERIA: PRODUCTION AND CHARACTERIZATION OF CLONED T-HELPER CELL HYBRIDOMAS RESPONSIVE TO RICKETTSIAL ANTIGENS, Thomas R. Jerrells, Carole J. Hickman, and Daniel L. Jarboe, Walter Reed Army Inst. of Res., Washington, DC. Immunity to Rickettsia species, obligate intracellular bacteria, involves two aspects of the T-helper cell: production of effector molecules, such as gamma interferon, and interaction with B-cells for production of antibody to T-dependent antigens. In the present study T-cell hybridomas were made using T-cell lines from animals immune to rickettsiae (R. conorii and R. tsutsugamushi) to determine if these two functions are mediated by distinct populations of helper cells. A total of 7 hybridomas were isolated and cloned from two fusions. All were Thy 1.2^{+} and produced factors after stimulation with antigen in a MHC restricted system that induced the proliferation of the IL-2 dependent cell line HT-2. The hybrids could be grouped into those that were L3T4 positive or negative but the response to antigen did not differ in magnitude between the two groups suggesting that L3T4 is not required for antigen recognition by the T-cell receptor on the $L3T4^{-}$ cells suggesting the presence of high affinity receptors. The hybrids also differed in the lymphokines produced after antigen or mitogen stimulation. All produced a T-cell growth factor assayed with HT-2 cells but differed in the amount of gamma interferon produced. Also, one hybrid was identified that did not produce Il-3. We are currently testing the cells for the production of B-cell factors. The results of this study show that the immune response to the obligate intracellular rickettsiae results in the production of subpopulations of T-helper cells based on the requirement for L3T4 for antigen recognition and on the type of lymphokine produced after stimulation.

H 111 SEQUENCE ANALYSIS AND EPITOPE LOCALIZATION OF THE COMMON PATHOGENIC NEISSERIA ANTIGEN H.8, Thomas H. Kawula, Stanley M. Spinola and Janne G. Cannon, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.
Pathogenic Neisseria express an outer membrane protein, H.8, that is not present in related commensal species. This protein is recognized by a single, previously described monoclonal antibody (MAb). We cloned and sequenced H.8-encoding DNA from a group C Neisseria meningitidis strain. Using the DNA sequence we located the H.8 open reading frame and predicted the amino acid sequence. A probable processing site was located 17 amino acids downstream from the N-terminal methionine. The predicted signal sequence contains the consensus processing/modification sequence of known prokaryotic lipoproteins. The H.8 protein amino acid composition is unusually rich in alanine and proline. Computer analysis using two different programs to predict protein secondary structure revealed that the amino terminal half of the H.8 protein is almost entirely alpha helix. Further analysis of the H.8 clone using Bal31 deletions and λgt11 subcloning delimited the H.8 MAb binding domain to a 20 amino acid region near the predicted N-terminal end of the protein. This MAb epitope is composed of four imperfect repeats of the amino acid sequence ala-ala-glu-ala-pro.

H 112 INTERACTION OF MONOCLONAL ANTIBODIES (mAbs) WITH THE ENZYMATIC SUBUNIT (S1) OF PERTUSSIS TOXIN (PT), James G. Kenimer, James L. Cowell, Peter G. Probst, and K. Jin Kim, Division of Bacterial Products, OBRR, CDB, FDA, Bethesda, MD 20892.

In order to study the structure-function relationship of various epitopes on the S1 subunit of PT we have generated and characterized several mAbs specific for this subunit. Immunoblotting experiments show that these mAbs bind to the S1 subunit and not to the other subunits (S2-S5) of PT. The mAbs are divided into 3 groups based upon their ability to neutralize the effects of PT in an in vitro CHO-cell assay. One group (10143CX4, 10126D2X11C, and 10143C1X6B) neutralized PT. Another mAb (10156FX1) displayed weak neutralizing ability but acted synergistically with the mAbs in the first group. A third mAb (10124FX2X5) had no neutralizing ability and did not influence the neutralizing ability of the other mAbs. Competition ELISA assays demonstrated competition within each group but not between groups, suggesting that this panel of mAbs identifies at least 3 separate epitopes on the S1 subunit. Tryptic digestion of purified S1 subunit (MW 28,000) results in the reproducible generation of several fragments with apparent MWs of 2000 to 8000 daltons when analyzed by SDS-PAGE. Immunoblotting of these fragments reveals that all the mAbs react with a major fragment of MW 7000 with several of the mAbs showing reactivity with smaller fragments. We are currently using gas-phase microsequencing of these immunoreactive tryptic peptides to determine the amino acid sequence of the epitopes.

H 113 MOLECULAR CLONING OF HAEMOPHILUS INFLUENZAE TYPE B OUTER MEMBRANE PROTEIN P1, Robert Munson, Jr., Washington University School of Medicine, St. Louis, MO, 63110

Outer membrane protein (OMP) P1 of Haemophilus influenzae type b was solubilized with sodium deoxycholate/0.2M NaCl and purified to homogeneity over DEAE Sephacel and CM Sepharose. The purified protein has an apparent molecular weight of 35,000 on SDS-PAGE when samples were prepared at room temperature and an apparent molecular weight of 50,000 when samples were prepared by heating at 100C. Polyclonal sera were generated in rabbits against the purified protein. αP1 antibody was reactive with surface-exposed epitopes of OMP P1 as determined by whole cell radioimmune precipitation and passive protection of infant rats challenged with type b Haemophilus. A genomic library of Haemophilus DNA was prepared in the lambda vector EMBL3. Plaques were screened with αP1 and immunologically reactive plaques purified. Synthesis of recombinant P1 was verified by Western blot of extracts of λP1 infected cells. The recombinant clones contained a 15kb SalI insert. The SalI fragment was cloned into the plasmid vector pcos2EMBL (Poustka et al., 1984) and transformed into E. coli strain HB101. No immunologically detectable P1 was synthesized by this plasmid, suggesting that OMP P1 expression in λP1 was dependent on a lambda promoter. A 6kb BamHI fragment was then subcloned into the T7 expression vector pAR2529 (Studier and Moffatt, 1986) and transformed into E. coli strain BL21(DE3). Protein reactive with αP1 was detected by dot blot. Synthesis of the protein is under the control of the T7 promoter. Thus, we have purified outer membrane protein P1 from Haemophilus influenzae type b and have cloned the structural gene for this protein.

H 114 LEGIONELLA PNEUMOPHILA SURFACE PROTEIN INHIBITS MYELOPEROXIDASE MEDIATED PROTEIN IODINATION IN HUMAN GRANULOCYTES. Alex Perry, N. Cary Engleberg, Stanley Holt, and Barry I. Eisenstein. The University of Michigan, Ann Arbor, MI 48109 and The University of Texas Health Science Center, San Antonio, TX 78284.

We found that a previously undescribed, surface located protein of Legionella pneumophila (LP) inhibits stimulation of Myeloperoxidase mediated protein iodination (MMPI) in human granulocytes. LP cells treated with hyperimmune anti-LP sera stimulated granulocytes poorly as compared with E. coli or L. micdadei bacteria treated with specific hyperimmune sera. However, the MMPI that was induced by LP-anti-LP was increased four-fold after the LP cells were either boiled for 20 minutes or blended, suggesting that such treatment removed an LP-surface-associated MMPI inhibitor. Blended or boiled LP supernatants inhibited stimulation of MMPI in granulocytes by a variety of particulate stimuli. Generation of superoxide or H_2O_2, however, were not inhibited by LP-supernatants. Pretreatment of the granulocytes with LP-supernatants did not result in inhibition of MMPI, thus the inhibitor does not act as a classic toxin. Preliminary data suggest that the material inhibits degranulation.

H 115 PROTECTIVE EFFICACY OF MONOCLONAL ANTIBODIES TO GROUP B STREPTOCOCCI (GBS) IS A FUNCTION OF EPITOPE DENSITY ON THE BACTERIAL SURFACE S.H. Pincus, H.R. Hill, and A.O. Shigeoka. Departments of Pediatrics, Internal Medicine and Pathology, University of Utah School of Medicine, Salt Lake City, Utah, 84132

We have produced and characterized a series of 11 monoclonal IgM antibodies to types I, II, and III GBS. Two antibodies react with carbohydrate antigens present on all GBS (two different epitopes detected), one antibody reacts with Ia carbohydrate, one detects a protein on all type I and II GBS, and the rest detect a sialated carbohydrate determinant on type III GBS. The protective efficacy of these antibodies has been assessed in a neonatal rat model of GBS sepsis. Antibodies to group B determinants are considerably less protective than those to type III carbohydrates. We have used direct binding ELISA, competitive inhibition, and radioimmunoassay to characterize the density on the bacterial surface of the epitopes recognized by each of these antibodies. The results demonstrate that on the surface of intact type III GBS there is at least a 5-10 fold greater density of the type determinant than the group determinant. Several type III GBS failed to demonstrate any binding of the group specific monoclonals to the surface of the intact bacteria. We have obtained from others a (non-protective) antibody to a non-sialated type III specific carbohydrate antigenic determinant. With this, we have confirmed that the difference between protective and non-protective antibodies is the number of antigenic sites on the surface of the bacteria.

H 116 CHARACTERIZATION AND CLONING OF THE DISULFIDE-CROSS-LINKED MAJOR OUTER MEMBRANE PROTEIN OF *LEGIONELLA PNEUMOPHILA*, F. D. Quinn, C. A. Butler, and P. S. Hoffman, University of Tennessee, Memphis, TN 38163.

All examined species of *Legionella* possess disulfide-cross-linked outer membrane proteins (OMP) that are serologically cross-reactive and have a monomeric molecular weight of 28,000. Amino acid analysis of the 28 kDa OMP of *L. pneumophila* reveals three cysteine residues and an amino acid composition comparable to porin proteins from other bacterial species. The N-terminal sequence is particularly hydrophobic. Adherence of selected species of *Legionella* to HeLa cells appears to correlate with relative levels of OMP. In addition, polyclonal antibody raised to the 28 kDa OMP blocks attachment of *L. pneumophila* to the HeLa cells by 80%. The gene encoding the OMP monomer was isolated from a Lambda 47.1 genomic library. Examination of lysates of *E. coli* NM538 by SDS-PAGE and immunoblot revealed expression of a polypeptide of similar molecular weight that reacted with anti-OMP serum. Moreover, the protein exhibited disulfide-cross-linking properties similar to those of the native protein. Lysates of *E. coli* infected with either wild-type Lambda 47.1 or recombinant phages containing random chromosomal insertions expressed no proteins reactive with the anti-OMP serum. Further study of the cloned gene in *E. coli* should provide additional evidence to show that the 28 kDa OMP, which is common to the genus, is involved in attachment and possibly in invasion.

H 117 EVIDENCE THAT THE PROTECTIVE CAPSULE (ETZ) AROUND PHAGOCYTIZED MYCOBACTERIUM AVIUM IS FORMED AS A RESULT OF THE PHAGOSOMAL ENVIRONMENT.

Ryter,A., Frehel,C., Bénichou, J.C. and Rastogi,N. Institut Pasteur. Paris. France.

In a previous EM study we showed that M.avium phagocytized by macrophages were surrounded by a thick electron-transparent zone (ETZ). This capsule plays an important role in protecting the bacteria against lysosomal enzymes. It was never obvserved in case of non-ingested bacteria. As glutaraldehyde fixation is known to cause collapsing of certain bacterial glycocalyx, we have compared in this communication the ETZ around M.avium before and after phagocytosis by the freeze-substitution method. ETZ was not observed around non-ingested bacteria but presented its usual appearance inside phagosommes, which confirmed the decisive role of phagosomal environment in ETZ formation. Antibody-coating experiments performed with the non-ingested M.avium in the test-tube provoked the formation of a ETZ-like capsule around the bacteria, irrespective of the fixation methods used.

H 118 INTERACTION OF THE ACTINOMYCES FIMBRIAL LECTIN WITH POLYMORPHONUCLEAR LEUKOCYTE RECEPTORS. Ann L. Sandberg, Michael J. Brennan, John O. Cisar, Richard A. Joralmon and Stephan E. Mergenhagen, NIDR, NIH, Bethesda, MD 20892.

Actinomyces viscosus T14V (T14V) and A. naeslundii WVU45 (WVU45) are phagocytosed by polymorphonuclear leukocytes (PMN) in the absence of antibodies and complement. Both bacteria possess type 2 fimbriae that are associated with lectin activity and T14V also has type 1 fimbriae. The possible participation of these fimbriae in PMN mediated destruction of the bacteria was assessed by colony counts of reaction mixtures containing sialidase, an enzyme produced by the actinomyces that, presumably, exposes PMN receptors for the bacteria. A mutant of T14V that possesses only the type 2 fimbriae was destroyed as effectively as the parent strain. In contrast, mutants of T14V and WVU45 lacking the type 2 fimbriae were not killed. Bactericidal activity was inhibited by lactose and β-methylgalactoside but not cellobiose or α-methylgalactoside. The lectin from Bauhinia purpurea (BPA) that reacts with β-Gal/GalNAc and the peanut agglutinin (PNA) that is specific for Galβ3GalNAc also inhibited killing whereas lectins reactive with other saccharides were ineffective. Radiolabeled BPA and PNA detected bands of 110, 85 and 78 Kd on sialidase treated nitrocellulose transfers of PMN extracts separated by SDS-PAGE. Radiolabeled WVU45 bound to several bands on thin layer chromatograms of partially purified PMN gangliosides including those that comigrated with purified GM1 and GD1b, both of which have Galβ3GalNAc termini and, after sialidase treatment of the chromatograms, with GD1a which contains NeuAcGalβ3GalNAc termini. Thus, phagocytosis of the oral actinomyces may result from the recognition of glycoprotein and glycolipid receptors on PMNs by the lectin associated with the type 2 fimbriae of these bacteria.

H 119 OUTER MEMBRANE PROTEINS OF CHLAMYDIA TRACHOMATIS ARE DEVELOPMENTALLY REGULATED. Lisa Sardinia and Don Ganem. University of California, San Francisco, CA, 94143

Chlamydia trachomatis is an obligate intracellular procaryote which lacks the typical cell wall of other procaryotes; structural stability is provided by extensive cross-linking of cysteine-rich outer membrane proteins. The interaction of chlamydia with host cells which leads to the internalization of these parasites has not been defined, however the outer membrane proteins must certainly be involved in this process. Cysteine-rich outer membrane proteins (12.3 kd and 58 kd) of the murine strain of C. trachomatis (MoPn) are not synthesized until late in infection (18 hpi) when reticulate bodies (RBs) have begun to redifferentiate to elementary bodies (EBs). An inhibitor of DNA synthesis (hydroxyurea) blocked synthesis of these proteins, but did not block protein synthesis early in infection. To analyze these developmentally regulated genes, antibodies were prepared against partially purified EBs. These antibodies recognized outer membrane proteins by Western blotting and radioimmunoprecipitation. The antibodies were used to screen a library of chlamydial DNA. Several clones were identified including a clone that directed the synthesis of a 70 kd protein. This protein was detected in Sarkosyl-precipitated outer membranes prepared from MoPn EBs that were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. The temporal control of this protein is under investigation.

H 120 COMPARISON OF INITIAL,COMPLEMENT-MEDIATED KILLING OF SALMONELLAE IN VIVO BY PERITONEAL MACROPHAGES IN Ity^R AND Ity^S MICE by H.Saxén,A.Muotiala and P.H.Mäkelä.Public Health Institute,Helsinki.Finland.

We have earlier shown that smooth O-6,7 Salmonellae-which are efficient activators of alternative complement pathway-are rapidly (within 30 min.)and efficiently (>90% of the challenge) killed by peritoneal macrophages.Isogenic O-4,12 transductants,whose rate of C3b binding is slower are clearly more resistant to this killing.

The Ity locus of the mouse chromosome 1 regulates the microbicidal capacity of murine macrophages during the first days of experimental salmonellosis.Therefore,the purpose of the present paper was to study whether this initial,complement-mediated killing is under Ity control.Therefore,approximately 10^6 O-6,7 Salmonellae were injected intraperitoneally to either Ity^R (CBA) or Ity^S (Balb/c, C57Bl/6) mice.The killing was recorded 60 minutes later by determining the numbers of viable bacteria in the peritoneal washings of the challenged mice.The killing turned out to be identical in all of the mouse strains.The intrahepatic killing in both Ity^R and Ity^S mice was determined from tissue homogenates at days 1.,3. and 4. after challenge and this killing was-as expected-more efficient in the Ity^R mice.

These experiments suggest that the initial,complement-mediated killing by peritoneal macrophages in situ is not under Ity control-in contrast to that in the liver macrophages.

H 121 THE 65KD ANTIGEN OF MYCOBACTERIUM TUBERCULOSIS. Thomas M. Shinnick, Centers for Disease Control, Atlanta GA 30333.
A 65,000 dalton protein has been identified as one of the medically-important antigens of M. tuberculosis. Antibodies and T-cells directed against this antigen have been detected in sera from tuberculosis patients. The gene encoding this antigen was isolated from a lambda gt11-M. tuberculosis recombinant DNA library using monoclonal antibodies directed against the 65KD antigen as the specific probes. The nucleotide sequence of this gene was determined and a 540 amino acid sequence deduced. The sequence was shown to correspond to that of the 65KD antigen by constructing a plasmid in which this open reading frame was fused to the lacZ gene. The resulting fusion protein reacted specifically with the anti-65KD antibodies. Unlike most mycobacterial transcriptional and translational signal sequences, the signal sequences upstream of the 65KD antigen coding sequence do function efficiently in E. coli to allow expression of this gene. The amino acid sequence of this antigen is very highly conserved between two distantly related mycobacteria, M. leprae and tuberculosis. The regions of this antigen that interact with T-cells and monoclonal antibodies directed against it have been mapped onto the primary amino acid sequence. As expected, the antibodies that bind the 65KD antigens of both M. tuberculosis and M. leprae react with shared sequences while the species-specific antibodies react with unique sequences.

H 122 SUSCEPTIBILITY OF NEISSERIA GONORRHOEAE TO KILLING BY NORMAL HUMAN SERA (NHS) IS CLOSELY LINKED TO CHANGES IN LIPOPOLYSACCHARIDE (LPS) STRUCTURE, WHILE BLOCKING OF SERUM KILLING IS INFLUENCED BY STRUCTURE OF PROTEIN I (PI), David S. Stephens, William M. Shafer, Carlos del Rio, Chandrasekhar Sankaran, Anne M. Whitney, VA Medical Center and Emory University School of Medicine, Atlanta, GA, 30303

The molecular mechanisms whereby gonococci develop resistance to killing by NHS are incompletely defined. We used the serum resistant (> 90% survival at 120 min) gonococcal strain FA19 and newly constructed isogenic transformants of this strain which differ in either LPS or PI structure to study the role of these gonococcal surface components in serum susceptibility. FA19 is known to contain the serum-antibody-complement genetic loci sac-1 and sac-3. All serum resistant transformants (8/8) had identical LPS molecular mass (M_R ~ 5,800), reacted with LPS monoclonal antibody L8, but differed in PI serovar [(IB9 (3/8) or IA1 (5/8)]. Serum sensitive (0% survival at 30 min) or serum intermediate transformants (< 10% survival at 120 min) differed in LPS molecular mass (M_R - 6,600 and - 6,200 respectively) and did not react with Mab L8. The serum sensitive and serum intermediate phenotypes all had the PI serovar IB9. FA19 and all transformants were killed by normal rabbit sera. When the 8 transformants which resist killing by NHS were studied in a blocking assay, killing of the IA1 but not the IB9 transformants by normal rabbit sera was blocked by NHS. These data suggest that both LPS and protein I structure may influence susceptibility of N. gonorrhoeae to killing by NHS.

H 123 LIPOPOLYSACCHARIDE (LPS) UNRESPONSIVE MOUSE CELLS CAN BE ACTIVATED BY PERTUSSIS LPS, Barnet M. Sultzer and Raymond Castagna, State University of New York, Health Science Center at Brooklyn, 11203.

The lymphocytes and macrophages of C3H/HeJ mice are known to be relatively unresponsive to the activating effects of purified LPS endotoxins from enteric gram-negative bacteria. More specifically, the lipid A component, which is responsible for the B-cell mitogenic and macrophage stimulating properties of the LPS, fails to turn on C3H/HeJ cells. However, the immunoncompetent cells of these mice are not deficient in the global sense, since they do respond to stimulation by selected proteins (EP) normally associated with LPS endotoxin in the bacterial outer membrane.

Recent comparative studies in our laboratory on the immunobiological activity of EP derived from Bordetella pertussis have revealed that the pertussis LPS has the property of activating C3H/HeJ cells as well. The purified LPS has no detectable protein (Lowry) and when enriched for its lipid component is inhibited by polymyxin B. This finding suggests that the lipid of the pertussis LPS contributes to the activation of C3H/HeJ cells and sets it apart from the lipid A of enteric LPS. Furthermore, the separation of the two major molecular species of pertussis LPS by hydroxylapatite chromatography has shown that the first species or the slower migrating component as detected by gel electrophoresis is responsible for most, if not all of the mitogenic activity for C3H/HeJ splenic lymphocytes.

H 124 ACTIVATION OF HUMAN BASOPHIL HISTAMINE RELEASE BY E. COLI ISOLATES, L.L. Thomas, S.J. Arra, and B.K. Karns, Rush Medical College, Chicago, IL. 60612

In the course of ongoing experiments, we observed that a clinical isolate of E. coli stimulated histamine release from human leukocytes. We have characterized this effect. Incubating leukocytes with 10^6 to 10^8 CFU/ml of the isolate for 30 min at 37°C resulted in a concentration-dependent release of up to 90% of the basophil histamine content. Leukocytes of all donors tested (n=25) responded with comparable sensitivity. Heat-inactivation, formalin-inactivation, or sonication of the E. coli abrogated the ability to stimulate release. That the E. coli stimulated histamine release by a direct effect on basophils was confirmed using preparations of purified (98%) human basophils. The release was Ca^{2+}-dependent and temperature-dependent; half-maximal release was obtained at 17°C and maximal release at 37°C. Kinetic studies revealed a concentration-dependent 5 to 15 min lag in onset of release, after which release increased with time for 20 to 30 min; release was stopped by the addition of EDTA. An additional 27 E. coli isolates were evaluated for ability to stimulate histamine release; 14 isolates exhibited activity at 10^6 to 10^8 CFU/ml and 13 isolates had no effect. No correlation was observed between the ability of the 28 isolates to stimulate histamine release and induce mannose-sensitive agglutination. However, an absolute correlation existed between ability to stimulate histamine release and hemolysin production by the isolates. It is postulated that the described effect may contribute to the pathophysiological changes observed in E. coli-associated septic shock.

H 125 MAINTENANCE OF BIOLOGICAL ACTIVITY IN RADIOIODINATED PERTUSSIS TOXIN, Gregory J. Tyrrell, Glen D. Armstrong, and Mark S. Peppler, University of Alberta, Canada.

We have developed a method to produce radioiodinated pertussis toxin which retained its activity in the goose hemagglutination and CHO cell assay systems. The procedure employed fetuin coupled to agarose to prevent inactivation of the toxin during the iodination reaction. PT which was labeled in the absence of fetuin lost virtually all of its ability to agglutinate goose erythrocytes and change the morphology of CHO cells. PT labeled by the fetuin technique bound to CHO cells significantly better than PT labeled in the absence of fetuin. Analysis of the PT iodinated in the presence of fetuin by affinity chromatography on fetuin- and wheat germ agglutinin-agarose and SDS polyacrylamide gel electrophoresis (SDS-PAGE) indicated minimal amounts of labeled fetuin or other contaminants in the preparations. 80 to 90% of the radioactivity was precipitated with 10% trichloroacetic acid, and SDS-PAGE demonstrated that all five of the toxin's subunits were labeled by the procedure. Although fetuin competed for binding of PT to goose erythrocytes it had no effect on PT activity in the CHO cell assay. Therefore, PT may use different receptors in these two cell types. PT may posses two receptor binding sites, or one site which has a different affinity for fetuin or goose erythrocyte receptors and CHO cell receptors. The labeling method will facilitate further investigations into the nature of pertussis toxin's interaction with these and other receptor systems.

H 126 IN VIVO KILLING OF E.COLI 018:K1 BACTERIA IN THE PERITONEAL CAVITY OF MICE. Jaana Vuopio-Varkila and P. Helena Mäkelä, National Public Health Institute, 00280 HELSINKI, FINLAND.

We are interested in the mechanism of the increased natural resistance to infection that develops in response to a sublethal infection or injection of lipopolysaccharide. Here we investigated the antibacterial capacity in the peritoneal cavity of mice 1 and 5 days after sublethal infection with live E.coli 018:K1. Increased resistance to rechallenge with an otherwise lethal dose of the same bacteria was observed already 1 day after the sublethal infection; at this time the resistance was expected to be due to nonspecific activation of the phagocytic cells since an antibody response would hardly have had time to develop. By contrast, an antibody response was expected to be present on day 5. Controls included naive mice as well as mice passively immunized with anti-K1-specific antiserum (Horse 46 from J.B. Robbins, NIH).

^{35}S-Met-labeled E.coli 018:K1 were injected intraperitoneally in the four groups of mice. The numbers of viable bacteria as well as the cpm were measured in peritoneal washings collected 30 or 60 minutes after the injection, and the percentage of the label associated with peritoneal cells was determined. Both the anti-K1-serum treated and day 5 convalescent mice eliminated almost all viable bacteria from the peritoneal cavity by 30 minutes and most of the label was cell-associated speaking for efficient opsonization by antibody followed by phagocytosis and killing of the bacteria. In the day 1 convalescent mice bacterial killing was less efficient and most of the label was found in the supernatant by 30 minutes. The proportion of the cell-associated label slightly increased with time. The effector cell responsible for the bacterial killing in the day 1 convalescent mice is being characterized.

H 127 AEROBACTIN IRON UPTAKE SYSTEM OF AEROBACTER AEROGENES 62-I, Virginia L. Waters and Jorge H. Crosa, The Oregon Health Sciences University, Portland, Oregon 97201

Although the aerobactin-mediated iron uptake system has been characterized genetically in E. coli (initially on the plasmid pColV-K30 but also as a chromosomal determinant), the siderophore aerobactin was chemically characterized as purified from culture supernatants of Aerobacter (or Enterobacter) aerogenes 62-I, a member of the Klebsielleae. We have cloned, mapped, and begun to characterize the relevant proteins of the plasmid-mediated aerobactin system of A. aerogenes 62-I. Published chemical data indicate that the siderophore aerobactin of E. coli is the same molecule as the aerobactin of A. aerogenes, but we have found that both the genes and the enzymatic proteins encoded by them have diverged considerably in the two systems. In contrast, the outer membrane receptor for ferric aerobactin of the systems show immunologic cross reactivity, are of the same molecular weight (74 kd), and are encoded by homologous DNA as found by Southern blot hybridization. The fact that the genes encoding the biosynthetic enzymes for the siderophores of the two systems share only one restriction enzyme site out of twelve sites mapped, while the essentials of the systems have been preserved, i.e., the siderophore itself and its outer membrane receptor, attests to the importance of this virulence factor across bacterial species.

H 128 A GENUS SPECIFIC CHLAMYDIAL ANTIGEN ELICITS OCULAR DELAYED HYPERSENSITIVITY IN IMMUNE GUINEA PIGS. Nancy G. Watkins, Abbie B. Moos, and Harlan D. Caldwell, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton MT 59840.

Chlamydia trachomatis is the etiologic agent of trachoma, a blinding disease which afflicts approximately 500 million people worldwide. We have been studying the immunopathogenesis of trachoma in a guinea pig model. In this model, a Chlamydia psittaci strain, GPIC, causes an acute conjunctivitis (guinea pig inclusion conjunctivitis) which is self-limiting and resolves without any apparent sequelae. However, ocular delayed hypersensitivity is induced during primary infection and can be elicited in previously infected animals by a Triton X-100 extract of viable chlamydial elementary bodies. The inflammation observed in the hypersensitivity response is clinically and histologically indistinguishable from that observed during primary infection. Immunity to chlamydial infection wanes rapidly (<6 months); whereas, hypersensitivity persists for a much longer period of time (>10 months). The chemical identity of the hypersensitivity antigen is unknown, although the antigen is heat-labile, sub-surface, and common to the genus Chlamydia. Hypersensitivity to a soluble chlamydial antigen may be an important component in the production of chronic inflammation which leads to the blinding sequelae of trachoma.

H 129 A POSSIBLE MECHANISM FOR THE HIGH AFFINITY BINDING OF ANTIBODY TO A POLYSACCHARIDE ANTIGEN, Michael R. Wessels, Alvaro Munoz, and Dennis L. Kasper, Channing Laboratory Harvard Medical School, Boston MA 02115, and Johns Hopkins School of Hygiene and Public Health Baltimore MD. Tritiated oligosaccharides derived from type III group B Streptococcus (GBS) capsular polysaccharide (CPS) were used to generate detailed saturation binding curves with a fixed concentration of rabbit antiserum in a radioactive antigen binding assay. A graded increase in functional affinity of antigen-antibody binding was observed as oligosaccharide size increased from 3 repeating units to 92 repeating units. The difference in affinity between the largest and smallest oligosaccharides tested was over 10^5-fold. Unexpectedly, the saturation binding values approached by the individual curves were inversely related to oligosaccharide chain length on a molar basis, but equivalent on a weight basis. This observation is compatible with a model in which binding of an immunoglobulin molecule to an antigenic site on the polysaccharide increases the affinity of adjacent antigenic sites for antibody binding. To test this hypothesis, the molecular size of immune complexes formed in the presence of antigen excess was estimated by gel filtration chromatography over Sepharose CL-6B. A population of type III GBS immune complexes eluted in the void volume of this column indicating the presence of complexes containing multiple antibody molecules per molecule of polysaccharide, rather than an antibody:antigen ratio of ≤ 1 as would be expected in antigen excess. These results support our hypothesis which predicts the formation of high molecular weight complexes, even in the presence of antigen excess.

H 130 ENTRY OF **C. TRACHOMATIS** IN NON-PROFESSIONAL PHAGOCYTIC CELLS IS BY RECEPTOR-MEDIATED ENDOCYTOSIS. Priscilla B. Wyrick and Rick L. Hodinka, University of North Carolina, Chapel Hill, N.C. 27514.

It has been assumed that **Chlamydia** spp. enter non-professionally phagocytic cells by a classical phagocytic maneuver. We believe, however, that these obligate intracellular parasites are internalized by an entry mechanism which resembles receptor-mediated endocytosis. Such a process could result in the internalization of chlamydiae into endocytic vesicles which are not destined to fuse with host lysosomes and, thereby, secure the survival of these organisms within infected cells. The entry of **C. trachomatis** into McCoy cells was studied morphologically by transmission electron microscopy. On adsorption of elementary bodies (EB) to host cells at 35°C, the EB were bound primarily to the surface of microvilli. They were also observed in coated pits located at the base of microvilli and along smooth surfaces of the host cells and were internalized within coated vesicles. Some EB were present in smooth-surfaced pits and vesicles. Individual EB were initially internalized into tightly bound vesicles. However, within 1-3 hr postinfection, EB-containing endosomes appeared to fuse with one another, leaving loosely bound vesicles containing multiple EB. This may explain the eventual appearance of only one large inclusion per infected host cell. We propose that **C. trachomatis** enters poorly phagocytic cells by a cellular process which closely resembles the adsorptive endocytosis of various hormones, proteins and viruses.

H 131 A YERSINIA PROTEIN WHICH CROSS-REACTS WITH AN ARTHRITIS-CAUSING HUMAN ALLOANTIGEN, David T.Y. Yu, Jin-Hai Chen, Dwight Kono and Juan Garcia Lobo, UCLA, Los Angeles, CA 90024.

It has been often been postulated that the reason why patients whose HLA typing is B27 develop arthritis after infection by Yersinia is because there is a bacterial component which mimicks this human HLA-B27 alloantigen. We have generated in Balb/c mice an IgM monoclonal antibody which reacts specifically with HLA-B27 lymphocytes. We labelled this antibody with ^{125}I and tested it against a panel of more than fifty different bacteria using both whole cell bacteria and bacterial envelopes. Strong reactivity was observed with two strains of Yersinia pseudotuberculosis. Ten monoclonal antibodies directed against other components on the human cells were not reactive. Using a modification of the preparative SDS-PAGE, we isolated the reactive Yersinia protein from the outer membrane of the bacteria. The apparent molecular weight was 23K. This isolated protein reacted with the anti-B27 antibody both by ELISA as well as by immunodiffusion. In a parallel series of experiments, a gene library was created from the Yersinia pseudotuberculosis using the plasmid pUC8 and the E. coli host HB101. The anti-B27 antibody was used for screening. A plasmid containing a 0.5 Kb inserted DNA was selected. The sequencing of this Yersinia gene might reveal to us the structural basis of the mimicry observed here.

Surface Interactions and Adherence: Pathogen Biology and Entry

H 200 ANALYSIS OF THE GENETIC AND STRUCTURAL DETERMINANTS OF THE D-MANNOSE-SENSITIVE ADHESION OF ESCHERICHIA COLI, Abraham, S.N., Goguen, J.D., and Beachey, E.H., University of Tennessee and VA Medical Center, Memphis.

The nature of the genes and gene-products mediating D-mannose-specific attachment of type 1 fimbriae of E. coli to eucaryotic cells was investigated by deletion mutation analysis of recombinant plasmid pSH2, which carries the genetic information for the synthesis and expression of functional type 1 fimbriae. Mutant p2004 was derived by a deletion remote from the structural gene encoding the 17kDA subunit protein of type 1 fimbriae. Phenotypically, the mutant demonstrated an eight-fold higher mannose-specific haemagglutination titer than the parent strain. On electron microscopy, the mutant strain expressed the same number of fimbriae as the parent strain. However, numerous 10 nm diameter rounded structures (fimbriosomes) were observed both closely associated with fimbriae and in the medium. Fimbriosomes isolated from the medium agglutinated yeast cells in a mannose-sensitive manner. These findings suggest that the enhanced adhesive properties of the mutant p2004 strain is associated with the production of fimbriosomes which may contain the determinants of the D-mannose-sensitive adhesion of type 1 fimbriated E. coli.

H 201 CHARACTERIZATION OF CLONED SEQUENCES FROM A UROPATHOGENIC STRAIN OF E. COLI CAPABLE OF CONFERRING MANNOSE SENSITIVE HEMAGGLUTINATION IN THE ABSENCE OF PILIATION.
Susan K. Amundsen, James L. Duncan, Anthony J. Schaeffer, and Scott J. Hultgren, Northwestern University Medical School, Chicago ILL. 60611. The ability of Escherichia coli to mediate mannose sensitive hemagglutination (MSHA) of erythrocytes and to bind to a variety of epithelial cells has been associated with the presence of type 1 pili on the bacterial cell surface. The relationship between the pilus structure however, and the adhesin responsible for hemagglutination is not clear. We have recently identified a clone (pHA9) resulting from insertion of a 12kb segment of DNA from a clinical isolate of E. coli into pUC19 which confers an MSHA-positive but non-piliated phenotype on strain ORN104 (minA minB Δpil recA13). Expression of the HA-positive phenotype appears to be regulated in response to environmental growth conditions, as transformants of pHA9 are MSHA-positive during growth on agar but MSHA-negative after growth in static or shaking broth. Minicell analysis of plasmid encoded polypeptides demonstrated that the loss of the HA-positive phenotype during growth in broth was accompanied by the loss of a 19kd polypeptide expressed during growth on agar. The sequences cloned in pHA9 have been tentatively mapped relative to recombinant plasmid pSH2 (pACYC184, pilABCDE hyp; Infect. Immun. 33:933) which contains the entire type 1 pilus operon from strain J96. DNA hybridization analysis suggested that although pHA9 lacks sequence homology to the pilin structural gene (pilA) and hyp, it shares homology with the remaining sequences in pSH2.

H 202 MECHANISM OF PHYTOHEMAGGLUTININ INDUCED BACTERIAL ASSOCIATION WITH RAT INTESTINE.
John Banwell, Richard Howard, Rixun Fang, Karen Anderson, Howard Ceri, Case Western Reserve University, Cleveland, Ohio and University of Calgary, Alberta, Canada.
Red Kidney bean lectin, phytohemagglutinin (PHA), dietary ingestion causes alteration in the intestinal microbial population density whereby certain components of the flora (E. coli, enterococcus) become adherent to the mucosa. The means by which PHA, which binds to the enterocyte membrane, might cause adhesion of this flora was studied by direct and indirect interaction of PHA with bacterial isolates. Four studies were performed: a) Morphological stabilization of the capsules of 9 isolates (4 streptococcus sp, 4 E. coli, straphylococcus sp) from PHA-fed rats.a)Bacterial pellets from 18 hour broth cultures were exposed to PHA or bacterial antisera and examined for EM stabilization of the glycocalyx, b) isolates in mid-log phase growth labelled with ^{35}S-methionine were examined for extent of binding to jejunal mucosal segments with/without PHA, c) agglutination of isolates in the presence of purified PHA or specific antiserum and d) binding of ^{125}I-radiolabelled PHA to 2 isolates with abd without BSA and fetuin was measured. Results demonstrated a) no morphological stabilization by PHA; stabilization occurred with $>10^3$ titer of specific antibody, b) binding of bacteria to jejunal segments was low>4.7% and unaltered by PHA c) only 1/9 isolates (staphylococcus sp) agglutinated weakly with PHA and d) ^{125}I-PHA to a staphylococcus sp and was inhibited by fetuin but binding to E. coli was non-specific. Conclusion. PHA associated bacterial adherence does not occur by the direct binding of the lectin with the bacterial glycocalyx.

H 203 ATTACHMENT OF PYELONEPHRITIS-ASSOCIATED PILI (PAP) TO THE CELL SURFACE OF E. COLI,
Lawrence B. Blyn, David A. Low, University of Utah, Salt Lake City Ut 84132.
Pathogenic E. coli capable of causing pyelonephritis in urinary tract infections (UTI) express specific pili (Pap pili) and adhesin proteins which allow the bacteria to adhere to the uroepithelium. Studies of the Pap pili in E. coli indicate that attachment of these pili to the cell surface of the bacterium requires a component in addition to the 21.5kd pilin monomer and two proteins required for pili assembly. Addition of a plasmid containing these gene products appears sufficient for assembly of pilin monomers into intact pili, however the pili do not attach to the cell and are subsequently secreted. Complementation with a DNA fragment located just downstream of the genes required to obtain assembled pili allows the pili to attach to the bacterial cell surface. A preliminary study of the DNA required for this complementation is presented.

H 204 DIRECT EXPRESSION OF BORDETELLA PERTUSSIS TOXIN SUBUNITS IN RECOMBINANT E. COLI, W. Neal Burnette,[1] Vernon L. Mar,[1] C. Fred Morris,[1] Kevin S. Marchitto,[2] Camille Locht,[2] and Jerry M. Keith,[2] Amgen, Thousand Oaks, CA 91320,[1] and NIAID, Rocky Mountain Laboratories, Hamilton, MT 59840.[2]

Vaccines of inactivated whole-cell Bordetella pertussis have provided an effective means of controlling whooping cough. Unfortunately, these vaccines have an unacceptably high incidence of side-effects. Lack of vaccine acceptance has led to a resurgence of disease in underimmunized populations; litigation associated with putative vaccine injuries has ultimately threatened the nation's vaccine supply and had a chilling effect on new vaccine research and development. To meet these challenges, we have taken a recombinant DNA approach to improvement of pertussis vaccine.

Pertussis toxin is a major virulence factor of B. pertussis and recent clinical evidence suggests that inactivated toxin alone may confer disease protection. We molecularly cloned portions of the toxin operon into bacterial expression vectors and obtained very high levels of directly-expressed (i.e., non-fusion) protein subunits in recombinant E. coli. The subunits, and various analogs, possess antigenic, immunogenic, and enzymatic properties of their authentic Bordetella-derived counterparts. These investigations demonstrate that techniques of genetic engineering can be used to produce pertussis components in heterologous cells at levels that are highly economical and which facilitate purification.

H 205 LOCAL HOMOLOGY WITHIN THE ACTIVE SITES OF DIPHTHERIA TOXIN AND PSEUDOMONAS AERUGINOSA EXOTOXIN A, Stephen F. Carroll and R. John Collier, Harvard Medical School, Boston, MA 02115.

Active site residues of two bacterial exotoxins, diphtheria toxin (DT) and Pseudomonas aeruginosa exotoxin A (ETA), have been identified by photoaffinity labeling with native NAD. Irradiating catalytically active toxin fragments and NAD with UV light induced efficient transfer (0.6-0.9 mol/mol) of the carbonyl moiety to a single glutamic acid residue in each protein (DT Glu-148 and ETA Glu-553). The ADP-ribosyltransferase and NAD-glycohydrolase activities of the photolabeled fragments were less than 2% that of unlabeled controls. When the amino acid sequences surrounding DT Glu-148 and ETA Glu-553 were compared, a high degree of local homology was observed. Within a stretch of 13 residues, 4 are identical (31%), and another 7 (54%) represent conservative substitutions know to occur in evolutionarily related proteins (85% total homology). Significant sequence alignments have also been detected in other regions of the catalytically active fragments. These results suggest that DT and ETA are distantly related, and that photoaffinity labeling with NAD may prove useful in identifying active site residues in other ADP-ribosylating or NAD-binding proteins.

H 206 ENDOGENOUS LECTINS: ASSOCIATION WITH PSEUDOMONAS INFECTIONS IN CYSTIC FIBROSIS, Howard Ceri and W. S. Hwang, Departments of Biology and Pathology, University of Calgary, Calgary, Alberta T2N 1N4

My laboratory has been interested in the role that may be played by host carbohydrate binding proteins (lectins) in the adhesion of pathogenic bacteria to mucosal surfaces. We have previously demonstrated the interaction of an endogenous rat lung lectin with the exopolysaccharide produced by Pseudomonas aeruginosa isolates from chronic lung infections seen in cystic fibrosis (CF). We now report the isolation of an endogenous lectin from human lung tissue, which closely resembles the rat lung lectin both in structure and in specificity. The lectin specifically binds Pseudomonas alginate in both agglutination inhibition assays and in ^{3}H-heparin binding sutdies. The lectin is present in the secretions at the ciliated surface and in the secretory Type-2 Pneumocytes of control lung sections. In CF lung the lectin visualized by immunohistochemistry is also seen in vacuoles present in the distended ciliated cells and in the secretory glands that underlie the mucosal surface. In patients suffering from bronchiectasis an increase in lectin secretion at the ciliated surfaces, and apical staining of the ciliated cells is seen, but much reduced from that described in CF tissue. There is also no staining of the secretory glands. The specificity of the human lectin, distribution at the mucosal surface, and increased presence in CF tissue all support the hypothesis of the involvement of endogenous lectins in these chronic CF infections.

H 207 RECEPTORS ON DIFFERENT ORAL SURFACES FOR THE ACTINOMYCES FIMBRIAL LECTIN. John O. Cisar, Michael J. Brennan, Floyd C. McIntire and Ann L. Sandberg. NIDR, NIH, Bethesda, MD 20892 and University of Colorado, Denver, CO 80262.

A lectin associated with the type 2 fimbriae of A. viscosus and A. naeslundii mediates the adherence of these gram positive bacteria to mucosal surfaces and certain strains of other bacteria which colonize tooth surfaces. The fimbrial lectin activity is strongly inhibited by Galβ3GalNAc and GalNAcβ3Gal and less effectively by galactose and N-acetylgalactosamine. The receptors on epithelial cells for the bacterial lectin are exposed by the action of sialidase and blocked by binding of the peanut agglutinin (PNA) which is specific for Galβ3GalNAc and the Bauhinia purpurea lectin (BPA) which recognizes this disaccharide and certain other Gal/GalNAc containing structures. Both plant lectins detect a glycoproteins of 160-Kd on epithelial cells. Mammalian cell surface glycolipids may also function as receptors since radiolabeled Actinomyces bind to gangliosides with Galβ3GalNAc termini and to globoside with terminal GalNAcβ3Gal on thin layer chromatograms. Receptors for the Actinomyces lectin on strains of Streptococcus sanguis are reactive with BPA but not PNA. Immunochemical studies have identified the receptor molecule on S. sanguis 34 as a polysaccharide composed of hexasaccharide units linked by phosphodiester bonds. Structural studies of the hexasaccharide have revealed an internal GalNAcβ3Gal which may account for the receptor activity of the polysaccharide. Thus, involvement of the Actinomyces fimbrial lectin in the formation of microbial communities on teeth and in the colonization of oral mucosal surfaces may depend on the recognition of structurally related receptors on streptococci and mammalian cells.

H 208 TRACHEAL CYTOTOXIN: A CONSERVED VIRULENCE DETERMINANT OF ALL BORDETELLA SPECIES. Brad T. Cookson and William E. Goldman, Washington Univ. School of Med., St. Louis, MO 63110

Bordetella spp. cause similar respiratory tract infections with different host specificity. Previously, we have shown that tracheal cytotoxin (TCT), a small peptidoglycan-derived molecule produced by B. pertussis, can mimic the primary cytopathology of these infections: specific destruction of the ciliated cell population. Other released toxins (such as pertussis toxin or extracytoplasmic adenylate cyclase) are not able to generate this damage, nor are those toxins produced by all non-pertussis species. To determine if TCT could be responsible for the common cytopathology in Bordetella infections, we have developed a rapid analytical method for detecting TCT. Strains of B. pertussis, B. avium, B. bronchiseptica, and B. parapertussis were grown to mid-log phase and the culture supernatants were concentrated using solid-phase extraction. Reversed-phase HPLC of these extracts revealed a common peak corresponding to conventionally purified TCT. Identity was confirmed with both triethylamine- and trifluoroacetic acid-buffered mobile phases. This suggests that a single molecule, common to all Bordetella species, may be responsible for the unique respiratory tract cytopathology associated with Bordetella infections.

H 209 A FIBRONECTIN DEGRADING PROTEASE FROM STREPTOCOCCUS PNEUMONIAE. Harry S. Courtney* and Edwin H. Beachey. Veterans Administration Medical Center and the University of Tennessee, Memphis, Memphis, Tn.

The only reported protease from Streptococcus pneumoniae is one that cleaves IgA1 but has no known effect on other serum components. We now report studies on a pneumococcal protease that degrades fibronectin. This protease(s) is excreted into growth media and is also extractable from cell pellets with 8M urea. Estimates of the MW of the enzyme(s) were made by electrophoresis of crude extracts of pneumococci in a SDS-PAG under reducing conditions and then electrophoretically transferring the samples into an SDS-PAG containing gelatin co-polymerized with the acrylamide. The gels were stained with coomassie blue and destained. The zones of clearance were presumed to be due to proteolytic cleavage. Three major zones of clearance were found correlating to MW estimates of 38, 44, and 100 kDa. The effect of inhibitors and pH were determined by assaying for release of radiolabeled fragments from (^{3}H)-fibronectin cross-linked to Sepharose 6B. The protease is optimally active under alkaline conditions and is inhibited by phenylmethyl sulfonyl fluoride, but not by EDTA, mercaptoethanol, maleimide or SDS. In addition, this protease(s) appears to degrade IgM, elastin, and albumin as determined by electrophoresis of radiolabeled proteins or by zones of clearance in acrylamide gels co-polymerized with these protein substrates. These results indicate that pneumococci produce a protease(s) that degrades several tissue proteins and may, therefore, represent a previously unrecognized virulence determinant.

H 210 RICKETTSIAE AS FACULTATIVE EXTRACELLULAR PARASITES, Gregory A. Dasch, Naval Medical Research Institute, Bethesda, MD 20814-5055.

Rickettsiae are generally viewed as obligate intracellular bacteria which exploit energy rich substrates found in the cytoplasm of their host cells such as mono-, di-, and triphosphate nucleotides and UDP-glucose. However, their relationship with the host cell may be more complex than indicated by this model. Although some rickettsiae like Rickettsia prowazekii and R. tsutsugamushi are released from infected host cells primarily by cell rupture, R. typhi and most spotted fever group rickettsiae accumulate in appreciable numbers in the fluid medium of infected cultures of Vero or L929 cells. The released rickettsiae are catabolically active and nearly all viable. The kinetics and extent of accumulation of different rickettsiae in the medium had no relationship to their capacity to form plaques in several cell types or to the rapid cytotoxic effects of some of the more virulent strains of R. rickettsii and R. conorii. Some non-pathogenic spotted fever rickettsiae did not accumulate in significant numbers extracellularly but this may reflect only their relatively limited intracellular replication. Fluid phase rickettsiae were easily purified in sufficient quantity to develop a rapid new method of strain and species typing of spotted fever group rickettsiae which uses SDS-PAGE and Western blotting. Two new species of African spotted fever group rickettsiae and five variant forms of R. conorii were identified by this approach. Besides the well-known dependence of rickettsiae on intracellular metabolites for growth, it is possible that replication of some rickettsiae is dependent on or greatly enhanced by their utilization of compounds found primarily in the extracellular milieu during their rapid extracellular transit between cells.

H 211 STEPS TOWARD THE MOLECULAR ANALYSIS OF ADHERENCE OF S. AUREUS. Lisa M. Dunkle, M.D. St. Louis University, St. Louis, MO 63104.

Molecular biological techniques have been applied to the study of adherence of S. aureus to human epithelial cells. An assay of adhesive capacity which quantitates adherence of ^{3}H-thymidine-labelled S. aureus to HeLa monolayers, expressed as Adherence Index (AI), has identified strains 1071 and 458 with AI of 60 and 10 respectively. *Transformation of plasmid* DNA purified from 1071 into 458 resulted in strain 458T$_3$ with AI of 45, indicating the presence of a plasmid-encoded adhesin. Growth in subinhibitory concentrations of several antibiotics has suggested that the adhesin associated with the plasmid gene is a protein; this is confirmed by a marked decrease in AI of 1071 and 458T$_3$, but not of 458, after brief digestion of whole bacterial cells with trypsin or pronase. Similar treatment of whole cells with lysozyme or endoglycosidase H did not decrease AI of any strain, mitigating against involvement of teichoic or lipoteichoic acids.

EcoRI fragments of the plasmid from 1071 ligated to pSC194 and transformed into 458 resulted in transformants of increased AI, but the specific fragment responsible could not be determined. The four individual EcoRI fragments have been cloned into E. coli HB101, using pBR325, but no gene expression detected. Ligation of these recombinant plasmids to an S. aureus vector is underway to isolate the fragments in S. aureus 458. The 6.8kb and 2.2kb fragments do not confer increased AI; the 7.6kb and 3.6kb fragments are pending.

Identification of a plasmid-mediated gene encoding an adhesive surface protein of S. aureus begins the molecular anaysis of staphylococcal adherence.

H 212 EVIDENCE FOR MANNOSAMINE AS AN OCULAR RECEPTOR MEDIATING P. AERUGINOSA ADHERENCE TO THE SCARIFIED EYE, Linda Hazlett and Richard S. Berk, Departments of Anatomy/Cell Biology and Immunology/Microbiology, Wayne State University, Detroit, MI 48201.

Recently, this laboratory has established that in vivo bacterial adherence of the opportunistic pathogen, P. aeruginosa (ATCC 19660) to the immature, 5 postnatal day (PND) old mouse ocular epithelium is mediated by a N-acetylneuraminic acid (NANA) receptor (Infect. Immun. 51: 697-699, 1986). In this model, the eyelids are sealed, ocular mucus is absent and there is no experimental trauma to the eye prior to bacterial challenge by infection beneath the fused eyelid. Current work has focused upon a similar analysis of the adult, 37 PND old animal model. The eye of the adult mouse, which exhibits surface mucus, was scarified by three-one millimeter incisions to the corneal surface with a sterile 26 g needle. The bacterial inoculum was mixed with either phosphate buffered saline (PBS, 1:1) or one of a panel of monosaccharides, the latter at a concentration of 25-40 mg/ml prior to topical application. A total volume of 5.0 ul containing 5.0 x 10^7 P. aeruginosa colony forming units was delivered to the scarified ocular surface. In the adult mouse, early in vivo bacterial adherence to the scarified eye was mediated by N-acetylmannosamine. Animals receiving N-acetylmannosamine treated bacterial inocula exhibited decreased ocular opacity at 24 h following infection when compared with all other infected mice. These qualitative data were corroborated by scanning EM studies which showed that the number of adherent bacteria was significantly reduced ($p \leq 0.001$) following treatment with N-acetylmannosamine when compared with organisms mixed with PBS or the other monosaccharides. Supported by EY 02986, 01935 and Core Vision Grant EY 04068 from the National Eye Institute.

H 213 Investigation of Bacterial Surface Components binding Fibronectin using Photoaffinity Labelling.

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A cleavable, iodinatable and photoreactive so called Double-Agent, SASD (Sulfosuccinimidyl 2(p-azidosalicylamido)-1,3'-dithiopropionate), attached to the 29 K fragment of fibronectin by acylation was found to bind specifically in the dark to Salmonella enteritidis, Staphylococcus aureus and Escherichia coli. The labelling of the bacterial components binding to the 29 K fragment of fibronectin was accomplished with the use of a strong light source, inducing the azidegroup of SASD to bind covalently to components near the reagent on the bacterial surface. The reagent was reduced with dithioerythritol to remove the 29 K fragment of fibronectin, and lysed bacteria were then run on a gradient polyacrylamide gel electrophoresis system. Autoradiography of the gels revealed bands at 20 K and 30 K for E. coli and at 30 K for S. enteritidis.

CHARACTERISATION OF NONFIMBRIAL ADHESINS OF UROPATHOGENIC E. COLI

H 214 Heinz Hoschützky, Thomas Moch and Klaus Jann
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Pathogenic E. coli bacteria adhere to eucaryotic cells by lectin-like adhesion proteins associated with the bacterial surface. These adhesins are either associated with fimbriae or form a protein capsule surrounding the bacterial cell as could be demonstrated by EM studies. Here we report on the characterisation of seven different nonfimbrial adhesins of uropathogenic E. coli bacteria. The adhesins were separated from the bacteria by heat treatment and purified to apparent homogeneity by differential ammoniumsulfate precipitation and high resolution anion exchange chromatography. In solution these adhesive proteins form high molecular weight complexes (10^6 D), the MWs of the subunits range from 13 - 28 kD. The isolated adhesins are different proteins by MW, IEP, peptide maps, carbohydrate specificity and immunogenicity. Ageainst four of these proteins sets of monoclonal antibodies have been prepared and it was possible to isolate antibodies with antiadhesive properties. So far we were not able to detect any immunological crossreaction with fimbrial subunits.

H 215 Cellular and Humoral Responses to M.pneumoniae Infections

Enno Jacobs, M. Drews, A. Stuhlert and W. Bredt, Freiburg University, FRG

M.pneumoniae causes upper respiratory tract infections and interstitial pneumoniae in man. The use of Western blotting methods has provided information about specific antibody responses to various protein antigens of the pathogen. The earliest and strongest antibody responses were found to a 168 kd protein, which is localized on the surface of the cell membrane and functions as an adhesin. Although IgG antibodies against this adhesin are found in the serum of most patients older than 20 years, reinfections with M.pneumoniae can occur in adults causing severe clinical symptoms.
Guinea pigs were infected intranasally with M.pneumoniae and examined for serum antibodies (IgM and IgG) as well as for secretory IgA antibodies in bronchial washings. The 168 kd protein was the major immunogen eliciting both serum IgG/IgM antibodies and a measurable IgA response in the lung. Additionall the specific proliferative response of lymphocytes from lung hilus lymphnodes was studied using M.pneumoniae whole cell antigen, crude lipid extract and purified membrane proteins respectively. The lymphocytes responded to all three types of antigen with significant proliferation. In additional experiments the effects of rechallenge will be studied.

H 216 IDENTIFICATION AND PURIFICATION OF THE LEGIONELLA HEMOLYSIN. Mark G. Keen and Paul S. Hoffman. Department of Microbiology and Immunology. University of Tennessee at Memphis, 38163.

Culture supernatants of many Legionella species exhibit both proteolytic and hemolytic activities. Hemolytic activity was found to be directly proportional to proteolytic activity in eighteen different Legionella isolates. Purification of the hemolytic activity from L. pneumophila supernatants utilizing DEAE-cellulose and Octyl-sepharose chromatography yielded a single protein band of 38kD on SDS-PAGE. This protein was determined to be the major neutral metalloprotease of L. pneumophila. The purified protein reproduced the hemolytic activity of concentrated supernatants in radial diffusion assays. Increasing amounts of purified protease increased zones of hemolysis. The hemolytic activity was inhibited by SDS or EDTA but not by PMSF or bovine serum. Analysis of hemolytic zones by SDS-PAGE showed substantial proteolysis. In addition, the purified protease is cytotoxic for cultured mammalian cells. These results indicate that this protease may be a factor contributing to the virulence of Legionella pneumophila.

H 217

Adhesion and Morphology of Escherichia coli type 1 Fimbriae: Three Genes, Encoding Minor Components are Required. Per Klemm and Gunna Christiansen. Technical University of Denmark and University of Aarhus, Denmark.

Three novel fim genes of Escherichia coli, fimF, fimG and fimH, have been characterized. These genes are not necessary for production of fimbriae, but are shown to be involved in the adhesive faculty as well as the longitudinal regulation of these structures. Complementation experiments indicate that both the major fimbrial subunit gene, fimA, as well as the fimH in combination with either one of the fimF and fimG genes are required for mannose-specific adhesion. The fimF, fimG and fimH gene products are likewise shown to play a major role in the fimbrial morphology, being longitudinal modulators; the amount of fimF, fimG and fimH proteins appears to control the length and number of the fimbriae, apparently by a purely statistical process. The DNA sequence of a 2050 base pair region containing the three genes has been determined. The corresponding protein sequences all exhibit homology with the fimbrial subunit protein.

H 218 STRUCTURE AND REGULATION OF P. AERUGINOSA PILIN GENES, Stephen Lory, Department of Microbiology SC-42, University of Washington, Seattle WA 98195.

The complete nucleotide sequence of the pilin genes of two independently isolated strains of Pseudomonas aeruginosa (PAK and PA103) has been determined. Comparison of the amino acid sequences deduced from the nucleotide sequences revealed clusters of highly conserved and highly variable amino acid regions along the pilin polypeptide. Genes from both strains encode a 6 amino acid N-terminus leader peptide (Met-Lys-Ala-Gln-Lys-Gly), which is not found in the mature assembled pilin. This polypeptide is part of an export signal sequence which includes a significant amino-terminal portion of the mature pilin polypeptide. The pilin polypeptides are nearly identical in the 60 amino acids which follow the leader peptide. The carboxyl terminal regions of the pilin polypeptides contain extensive regions of divergence in their amino acid sequences. A hydrophilicity analysis of the pilin polypeptides indicated that they are structurally similar, despite extensive differences in the primary structure of their carboxyl termini. The transcriptional initiation site of the pilin gene has been determined by S1 nuclease mapping. The regions located at -10 and -35 base pairs from the transcriptional initiation site show no significant homology to the consensus E. coli promoter, but some homology to the other Pseudomonas promoters. Further analysis of the sequence near the transcriptional initiation site showed striking similarity to the promoters of several positively regulated operons including those involved in nitrogen fixation, utilization, and uptake in Gram-negative bacteria.

TRANSFER OF PLASMID AND CHROMOSOMAL DNA BETWEEN B.FRAGILIS and E.COLI.

H 219 Michael H. Malamy and Francis P. Tally, Tufts University, Boston MA 02111

Several drug resistance plasmids with transfer factor properties have been described in B. fragilis. pBFTM10 is a self-transmissable 15 kb plasmid that contains transposon Tn4400 which codes for clindamycin-erythromycin resistance in B.fragilis. Transfer of pBFTM10 between strains of B.fragilis occurs at frequencies of 10^{-8}/input donor. Most tetracycline (tet) resistant B.fragilis strains contain a tet inducible transfer system which results in efficient (10^{-8}/input donor) transfer of tet resistance. Using strain TM230 and tet resistant transconjugants of this strain, we have demonstrated that the "tet transfer element" can promote transfer of chromosomal DNA to B.fragilis recipients, and can even result in transfer of Tn4400 from the chromosome of B.fragilis donors to E. coli recipients. Most E.coli transfer factors do not successfully transfer to B. fragilis recipients. Using the broad host range plasmid RP4, it has been determined that plasmid transfer occurs but that the plasmid is unable to replicate in the B. fragilis recipient cell. Shuttle vectors such as pGAT500 have been constructed consisting of plasmids capable of replicating in B.fragilis (pBFTM10) and plasmids capable of replicating in E.coli (the pBR322 derivative pDG5 of Don Guiney which also contains the oriT region of RP4). RP4 efficiently mobilizes these plasmids from E.coli to B.fragilis recipients. These plasmids transfer from B. fragilis donors to B.fragilis recipients. pGAT500 transfers from B.fragilis donors to E.coli recipients at low efficiency; but the "tet transfer element" greatly increases the transfer of these chimeric plasmids from B. fragilis to E. coli.

ISOLATION AND CHARACTERISATION OF THE ADHESIVE PROTEINS OF FIMBRIATED E. COLI

H 220 Thomas Moch, Heinz Hoschützky and Klaus Jann
Max-Planck-Institut für Immunbiologie, Freiburg, BRD

The adherence of pathogenic E. coli bacteria to host cell surfaces is mediated by lectin-like adhesive proteins associated with the bacterial surface. In many cases the adhesiveness of the bacteria is correlated with the expression of supramolecular extracellular structures called fimbriae. Genetic analyses of cloned fimbrial genes (S-, P-, and type I fimbriae) revealed that different chromosomal sites determine fimbration and adhesiveness. In spite of the wealth of information on fimbriae, the nature of the adhesins and their possible interaction with fimbriae has not yet been defined. Here we report on the separation of adhesins and fimbrial subunits of S-, P-, and SS142 fimbriae. In all cases analyzed so far, fimbriae and the corresponding adhesins are different proteins by MW, IEP and immunogenicity. By functional studies we could demonstrate that not the fimbrial subunit but the isolated proteins are mediators of the adhesive properties of fimbriated E. coli bacteria. These results are further supported by an antiadhesive monoclonal antibody directed against the S-fimbriae associated adhesin that does not crossreact with the fimbrial subunit.

H 221 EXPRESSION OF PROTECTIVE EPITOPES OF SEROTYPES 5 AND 24 GROUP A STREPTOCOCCAL M PROTEINS IN *SALMONELLA TYPHIMURIUM*. T.P. Poirier*, V. Burdett, M.A. Kehoe, and E.H. Beachey. VA Med. Ctr. and the Univ. of Tenn., Memphis, TN.; Duke Univ., Durham, NC.; and Univ. of New Castle upon Tyne, England.

M proteins expressed on the surface of group A streptococci enable the microorganisms to evade phagocytosis, an event which is easily overcome in the presence of M protein specific antibodies. M proteins have been cloned previously into *Escherichia coli* plasmids. In *E. coli*, the plasmid pMK207 expresses the entire M5 gene (*smp* 5) as a 56,000 kDa protein, while pVB41.1 possesses a *Sau* 3A fragment of the M24 gene producing a 17,000 kDa M polypeptide. Individually, these plasmids were used to transform the r^-, m^- *Sal. typhimurium* LB5000 strain. The *aro*$^-$ *Sal. typhimurium* SL3261, a strain developed for use as a vaccine-delivery vehicle in a BALB/c mouse model, was then transformed with purified, unmodified pMK207 and pVB41.1. Immunoblot analysis revealed that both *Sal. typhimurium* LB5000 and SL3261 expressed the M proteins the same molecular weight as in *E. coli*. In preparation for mouse protection studies, an LD_{50} was established for intraperitoneal infection of BALB/c mice by *Strep. pyogenes* M serotypes 5 and 24. We are currently investigating the potential of *Sal. typhimurium* SL3261 expressing M5 and M24 proteins to serve as the delivery vehicle for protective M5 and M24 epitopes either by intraperitoneal or oral immunization of BALB/c mice in order to protect against subsequent challenge with homologous M serotypes of *Streptococcus pyogenes*.

H 222 FIBRINOGEN BINDING COMPONENT OF STAPHYLOCOCCUS AUREUS, Lech M. Switalski and Magnus Hook. University of Alabama, Birmingham, AL 35294

Strains of Staphylococcus aureus clump in the presence of fibrinogen. This clumping is due to the presence of fibrinogen binding components on the surface of staphylococci. These components, refered here as a fibrinogen receptor have been isolated from a strain S. aureus Newman and further characterized. Isolation procedure involved digestion of bacteria with lysostaphin, absorption of receptors by affinity chromatography, followed by gel filtration. The fibrinogen receptor is a protein of apparent molecular weight of 62000, the amino acid analysis indicates high content of glutamic acid/glutamine, glycine and lysine. Purified fibrinogen receptor is highly immunogenic. One monoclonal antibody, out of several tested appears to recognize the fibrinogen binding site of the receptor and inhibits binding of fibrinogen to bacteria. The role of this antibody, as well as antibodies recognizing other parts of fibrinogen receptor in the inhibition of fibrinogen mediated binding of bacteria to surfaces will be discussed.

H 223 ATTACHMENT OF TREPONEMA PALLIDUM TO RABBIT AND HUMAN CELL MONOLAYERS, D. Denee Thomas, James N. Miller, and Michael A. Lovett, UCLA, Los Angeles, CA 90024. The association of virulent Treponema pallidum with rabbit and human cells in culture was examined in this study. Epithelial human cells (HEp-2 and HeLa), human neuroblastoma cells, and endothelial cells of both rabbit and human origin were tested for ability to bind intrinsically radiolabeled, motile T. pallidum. Attachment of treponemes to each cell type was linear over the six hour time period studied. Numbers of attached treponemes per cell were greatest for HeLa cells, followed by HEp-2, neuroblastoma, human umbilical vein, and rabbit aortal endothelial cells. Attachment of treponemes to HeLa cell monolayers was inhibited up to 70 % by preincubating T. pallidum with rabbit immune serum, and was inhibited by 40 % following preincubation of organisms with polyclonal rabbit serum directed against 4D, a treponemal surface molecule previously cloned and characterized by us. Mild trypsinization of attached treponemes resulted in release of treponemes without visible damage to the cell monolayer. Radioactivity associated with the cell monolayer following the trypsin treatment suggested that some T. pallidum had entered cultured cells.

H 224 MOLECULAR ANALYSIS OF THE T-DNA TRANSFER PROCES. Benedikt Timmerman, Scott E. Stachel, Marc Van Montagu, and Patricia Zambryski, State University Ghent, Belgium

Upon genetic transformation of plant cells by Agrobacterium tumefaciens, the T-DNA border sequences (25 bp repeats) are recognized and they direct, in a polar fashion, the mobilization of the T-DNA from the Ti plasmid of the bacterium to the plant nuclear genome (1). T-DNA transfer is triggered by the plant exudate acetosyringone which activates Ti plasmid encoded virulence genes (vir) (2). Vir gene products are instrumental to the actual DNA transfer. We have identified several T-DNA associated molecular structures which most likely represent the primary steps of the T-DNA transfer proces : the generation of a nick in the bottom strand of both T-DNA 25 bp repeats, and the formation of a free single-stranded, unipolar, linear T-DNA molecule (T-strand) (3). A third T-DNA associated event dependent on Vir induction is the precise excision of the T-DNA from the Ti-plasmid (4) and the formation of T-DNA circles (5, 6). The frequency of this event is ± 100 times lower than T-strand synthesis. The presented data suggest that excision occurs via site specific recombination, dependent on a single-stranded nick at one recombination site, while homology to this sequence at the other recombination site suffices. The role and relevance of these phenomena in the T-DNA transformation mechanism will be discussed.

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H 225 DYNAMICS OF CHLAMYDIAL ANTIGEN EXPOSURE ON INFECTED CELLS. Charles E. Wilde III, Terry McBride and Susan Karimi, Indiana University School of Medicine, Indianapolis, IN 46223.

A panel of monoclonal antibodies was utilized to study the fates of several chlamydial antigens following infection of various cell lines. Antibody 47A2 reacts with chlamydial LPS, antibody 1A5 reacts with MOMP of the L_2 serovar and antibody 54A4 reacts with several protein antigens including MOMP and the 60 kD protein. For a period of six hrs following inoculation of HeLa cells with the L_2/434/Bu serovar of C. trachomatis, a progressive decline in the accessibility of LPS on intact cells was measured by RIA. However, during this time period, a transient increase was seen in the exposures of 1A5- and 54A4-reactive epitopes. Total antigen reactivities in detergent lysates of infected cells did not change over this time period. The dynamic increase of cell-associated 54A4-reactive epitope did not occur at 4° or in the presence of dithiothreitol. In contrast, from 12 hrs through the end of the infectious cycle, indirect immunofluorescent (IIF) analysis at 4° or 22° of unfixed HeLa or McCoy cells infected with either the L_2 or F serovars indicated that LPS antigen could be detected on cell surfaces; the apparent amount of cell-surface LPS increased during this period. No surface-associated chlamydial protein antigens could be detected by IIF during this period. As previously demonstrated, a decline in cell membrane fluidity occurs concomitant with the increase in membrane-associated LPS. These data suggest that, during the infectious cycle, various chlamydial epitopes undergo dynamic changes in location with respect to host cell membrane components.

H 226 AF/R1 PILI OF ESCHERICHIA COLI STRAIN R 3C-1 PROMOTE COLONIZATION AND DISEASE BUT ARE NOT REQUIRED FOR IN VIVO ENTEROADHERENCE. M. Wolf, I. Pintado, G. Andrews, R. Sjogren, and E. Boedeker, Walter Reed Army Institute of Research. Washington, D.C. 20307. AF/R1 pili are plasmid encoded adherence factors which promote attachment of E.coli strain RDEC-1 to intestinal brush borders in a species specific manner, however their role in vivo is not well defined. To determine whether AF/R1 pili on RDEC-1 are essential for colonization, diarrheal disease, or enteroadherence we constructed mutant strain M34, an RDEC-1 derivative with a Tn5 insertion mutation in its 86 Md plasmid, which does not express pili and fed this to rabbits. M34 does not adhere to intestinal brush borders. Diarrhea occurred in 4/4 rabbits fed 10^6 RDEC-1, but in only 1/4 rabbits fed 10^6 M34. 4/4 animals fed 10^8 of either organism developed diarrhea. Persistent weight gain was seen only in animals fed 10^6 M34. All animals fed RDEC-1, and those fed 10^8 M34, lost weight as diarrhea developed. Survival was greater in animals fed M34 at either dose, than in those fed RDEC-1. Shedding of M34 was delayed by 2-3 days compared to RDEC-1 shedding in rabbits fed 10^6 organisms. Shedding after inoculation of 10^8 organisms was equivalent. One animal in each group was sacrificed at day 2 and day 7. Day 2 colonization of distal segments was 2-3 logs lower after 10^6 M34 as compared to RDEC-1. At day 7 after 10^6 organisms, M34 counts were 6 logs less than RDEC-1 in jejunum and ileum, but were similar in distal segments. Similar enteroadherence in cecum was seen in all animals regardless of the organisms inoculated. Conclusion: the ability to express AF/R1 pili in the parent organism promotes earlier colonization and development of disease and increases mortality in animals fed lower doses. Expression of AF/R1 pili does not seem to be required for in vivo enteroadherence.

H 227 CLONING AND EXPRESSION OF AN ACTINOMYCES VISCOSUS GENE ENCODING A SUBUNIT OF THE FIMBRIAE THAT MEDIATE ATTACHMENT TO THE TOOTH SURFACE. Maria K. Yeung , Bruce M. Chassy, and John O. Cisar. NIDR, NIH, Bethesda, MD 20892.

The type 1 fimbriae of Actinomyces viscosus T14V mediate adherence of this gram-positive organism to saliva-treated hydroxyapatite. This interaction is an initial event in the colonization of tooth surfaces and recent studies suggest the involvement of acidic proline rich proteins as specific receptors in the acquired salivary pellicle. Molecular cloning of the gene encoding a subunit of this fimbrial adhesin was undertaken and its product expressed in Escherichia coli examined. Chromosomal DNA from A. viscosus T14V was partially restricted with Sau3A I followed by cloning into E. coli JM109 using pUC13 as the plasmid vector. Two reactive recombinant clones were identified by colony immunoassays using specific polyclonal and monoclonal antibodies directed against the type 1 fimbriae of A. viscosus T14V. Immunoblot analysis revealed the expression of a 65-kilodalton protein that migrated slightly behind an antigenically similar protein from native fimbriae. By deletion analysis, the gene encoding the cloned protein was localized on a 1.9-kilobase-pair fragment. Isolation and purification of the cloned protein from the E. coli cytoplasmic fraction was achieved by ion-exchange, immuno-affinity and gel permeation chromatography. Rabbit antibodies prepared against the cloned protein and against purified type 1 fimbriae from A. viscosus T14V gave identical patterns in Western blotting of partially dissociated type 1 fimbriae and both inhibited bacterial adsorption to saliva-treated hydroxyapatite. Thus, the gene cloned encodes a subunit of the fimbrial adhesin.