MOLECULAR EVENTS IN MICROBIAL PATHOGENESIS

Organizers: Vincent Fischetti, Virginia Miller and Marian Neutra

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Surface Molecules on Pathogens Responsible for Invasion and Disease: Attachment and Entry

B 001 LISTERIA MONOCYTOGENES: MOLECULAR BASIS OF ENTRY AND SPREAD IN EPITHELIAL CELLS. Pascale Cossart. Laboratoire de Génétique Moléculaire des Listeria , Institut Pasteur , 28 rue du DR Roux, Paris. 75015. France.

Listeria monocytogenes, in contrast to other invasive bacteria, has the capacity to invade and spread in a wide variety of cell types *in vitro* as well as *in vivo*. During disease, as shown in the murine model, bacteria rapidly cross the intestinal barrier and main foci of infection appear in the liver, the organ where 90% of the bacterial load is found less than ten minutes after iv injection and the spleen (10%). Then, depending on the host response, bacteria will either be eliminated or will start to replicate and get to the brain and the placenta in the pregnant woman.

Our laboratory has taken a genetic approach to dissect the molecular interactions underlying the mechanisms of entry and identified the first listerial factor involved in entry in epithelial cells (1). This surface protein called internalin is encoded by *inlA* which when expressed in *L. innocua,* confers invasivity to this normally non invasive species. *InlA* is a member of a multigene family and *inlB*, the gene lying downstream *inlA* is part of this family. Our working hypothesis concerning this family is that other genes of the family could be involved in other cell type recognition. We have now shown that invasiveness strictly corrolates with expression of *inlA* which is maximal in exponential growth (2, 3). Internalin is not constitutively expressed on the bacterial surface and is found in high amounts in culture supernatants at stationary phase. This release starts during exponential growth. *InlB* mutants do not release internalin, nor do they express it on the surface. They are non invasive. We are currently investigating the role of *inlB* and addressing the role of the membrane-bound form of internalin versus the released-form in invasivity.

The intra- and inter-cellular spreading of *L.monocytogenes* relies on a mechanism of continuous directional actin assembly that propels the bacteria from one cell into the other through the formation of long protrusions that are phagocytosed by the neighbouring cell. This actin-based motility requires the expression of the bacterial gene *actA* (4). We have used immunocytochemistry to show that the *actA* gene product, ActA, is distributed asymetrically on the bacterial surface: it is expressed at one pole and is increasingly concentrated towards the other (5). This polarized distribution of ActA is linked to bacterial division: ActA is not expressed at the pole formed during the previous division. On intracellular bacteria, ActA is expressed at the site of actin assembly suggesting that ActA may be involved in actin filament nucleation off the bacterial surface. We predict that the asymetrical distribution of the protein predetermines the direction of movement in the direction of the non-ActA expressing pole. The main question now concerns the mode of action of ActA and its various ligands. Whether actin is one of them is central to the understanding of the process.

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B 002 CROSS-TALK BETWEEN *SALMONELLA* AND MAMMALIAN HOST CELLS, Jorge E. Galán, Department of Microbiology, School of Medicine, SUNY Stony Brook, Stony Brook, NY 11794-5222

Central to the pathogenicity of *Salmonella sp.* is their ability to enter mammalian cells. We have identified a genetic locus, *inv*, located at 59 minutes in the*Salmonella* chromosome which is required for efficient entry of these organisms into cultured mammalian cells. Sequence and functional analysis of this region, have identified at least 12 genes encoding proteins with homology to determinants involved in organelle assembly and/or surface presentation of proteins lacking typical signal sequences in other organisms. It appears that *Salmonella* entry involves the assembly of a supramolecular structure required for the surface presentation of determinants necessary to trigger host cell responses that lead to bacterial uptake.

Upon contact with non-phagocytic cells, *Salmonella* induces the formation of membrane ruffles at the point of bacteria/host cells interaction. Using high-resolution low-voltage scanning electron microscopy, we have found that contact with epithelial cells results in the transient assembly of appendages on the surface of *S. typhimurium*. Upon triggering internalization, the appendages are shed or retracted. Examination of the interaction of *S. typhimurium*. strains carrying mutations on different *inv* genes, has demonstrated that the transient assembly of these appendages is required for bacterial entry into cultured mammalian cells.

We have found that *Salmonella* internalization is accompanied by a marked rise in the levels of intracellular free calcium which is required for bacterial internalization. We have studied the host-cell signal transduction pathway that leads to *Salmonella* entry into Henle-407 cells. We have found that *Salmonella* internalization into these cells involves the activation of the epidermal growth factor receptor (EGFR). Activation of this receptor leads to a cascade of events that involves the activation of MAP kinase and phospholipase A_2, with the subsequent production of arachidonic acid and leukotriene D_4. This eicosenoid presumably participates in the opening of a calcium channel allowing the influx of calcium required for bacterial entry. We have also studied the signal transduction pathway that leads to bacterial entry into other cells, including those lacking EGFR. We have found that phospholipase $C\gamma$ activity with the production of inositol phospholipids and the generation of calcium fluxes are essential features of these other pathways.

B 003 COMPLEMENT RESISTANCE OF PATHOGENS, Rolf D. Horstmann, Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany.

The opsonic and cytolytic activities of the complement system are in the first line of mammalian defense against invading microorganisms. Via the alternative pathway, complement may be activated before the appearance of specific antibody.
Alternative pathway activation is initiated by the serum component C3, which, upon routine activation to C3b, is able to covalently attach to most if not all biological particles. If particle-bound C3b is not inhibited specifically, it forms the C3bBb complex, an enzyme that greatly amplifies the attachment of opsonic C3b and that initiates the assembly of the cytolytic membrane attack complex. Autologous cells are protected against complement damage by several inhibitory mechanisms, most of which act upon the C3bBb complex.
Pathogens which are able to survive in host tissues have adopted distinct strategies to evade complement attack. In most instances studied so far, the evasion strategies were found to mimick host regulatory mechanisms by affecting the generation or stability of the C3bBb complex. Such activities have been identified in a wide range of microbial pathogens including, for instance, Herpes simplex virus, *Streptococcus pyogenes*, and the protozoan *Trypanosoma cruzi*.
More recent data extend the spectrum of evasion strategies used by pathogens by showing an as yet unrecognized type of complement modulation by *Yersinia enterocolitica* 09 and an apparently nonspecific kind of resistance by the protozoan parasite *Entamoeba histolytica*, which seems to prevent complement-mediated lysis predominantly by an unusually high activity in basic cellular functions such as endocytosis and membrane shedding.

B 004 MOLECULAR MIMICRY AND *CHLAMYDIA* ATTACHMENT AND ENTRY INTERACTIONS WITH EUKARYOTIC CELLS, Richard S. Stephens, University of California, Berkeley, CA 94720

Chlamydia trachomatis is an obligate intracellular eubacteria that is an important human pathogen. Diseases caused by C. *trachomatis* are found in two major epidemiological settings—they are the leading cause of preventable blindness (i.e., trachoma) and they are the leading cause of sexually transmitted diseases. Little has been understood concerning the molecular basis of *Chlamydia*-eukaryotic host cell interactions that are required for infection. It has recently been proposed that chlamydiae have evolved a novel trimolecular mechanism of microbial attachment to mammalian host cells in which a molecular mimic of heparan sulfate is bound to infectious organisms and this complex is bound by a heparan sulfate receptor on host cells (1). For one biovariant (serovar L2), attachment and consequently infectivity of *C. trachomatis* to host cells is mediated by a heparan sulfate-like molecule bound to the surface of the organism. Evidence for this interaction is 1) attachment is inhibited by heparan sulfate and heparin but not by other glycosaminoglycans (GAGs), 2) receptor analogs for heparan sulfate bind chlamydiae and block attachment, and 3) heparan sulfate lyase (heparitinase) treatment of chlamydiae specifically hydrolyzes a ligand essential for attachment and infectivity. Direct evidence for the role of GAG in attachment of chlamydiae to host cells is that attachment can be restored for heparitinase-treated organisms by coating organisms with exogenous heparan sulfate. Moreover, the infectivity of these organisms is rescued. Similar data has been obtained for the other biovariants of *C. trachomatis*. Using mutant eukaryotic cell lines that cannot synthesize GAGs (2) a unique sulfated GAG species is synthesized only in chlamydia infected host cells. This is structurally and functionally related to the chlamydial adhesin ligand in that it is a sulfated GAG, it is sensitive to heparitinase digestion, and it can rescue infectivity of heparitinase-treated organisms. To determine if the chlamydial ligand is only involved in attachment or whether it is also important in subsequent entry events, the chlamydia-eukaryotic cell interaction has been modeled using latex microspheres coated with heparan sulfate or native chlamydial ligand. Both compounds coated on beads mediate attachment to cells that is competitively inhibited by chlamydia organisms; likewise, heparan sulfate coated beads competitively inhibits chlamydial infectivity. Significantly, coated beads are endocytosed as determined by a protease resistant bead fraction associated with viable cells that was time and temperature of incubation dependent. Cell endocytosis of coated beads was confirmed by visualization of internalized beads using electron microscopy. It is concluded from these studies that *C. trachomatis* synthesizes a molecular mimic of heparan sulfate and exploits natural eukaryotic pathways to mediate chlamydial entry into host cells.

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Intracellular Interactions Between Pathogens and Host

B 005 STRUCTURE AND FUNCTION OF THE PARASITOPHOROUS VACUOLE MEMBRANE (PVM) SURROUNDING *TOXOPLASMA GONDII* by K. A. Joiner[1], C.J.M. Beckers[1], D. Bermudes[1], P. N. Ossorio[1], J. C. Schwab[1], and J.F. Dubremetz[2], [1]Yale Univ. School of Med., New Haven, CT & [2]U42 Inserm, Villenueve d'Ascq, France

The obligate intracellular protozoan parasite *Toxoplasma gondii* invades and replicates within essentially all nucleated mammalian cells. *T. gondii* entry is a rapid event (<10 seconds) and depends upon the cytoskeletal apparatus of the parasite. The newly formed vacuole does not fuse with host cell endocytic compartments and does not acidify, likely as a consequence of formation during cell entry of a specialized PVM depleted of plasma membrane proteins from the host cell.

Despite the absence of fusion, the PVM does mediate interaction with the host cell. Shortly after parasite entry, host cell mitochondria and endoplasmic reticulum become tightly apposed to the PVM, as has also been observed with Legionella and Chlamydia. Association of mitochondria with the PVM is not dependent upon the host cell cytoskeleton, parasite viability or mitochondrial membrane potential, and may represent a protein-protein or protein-lipid interaction. In addition, a pore across the PVM permits rapid bi-directional exchange of molecules <1400d between the vacuole and the host cell cytoplasm.

The PVM is modified by parasite proteins from two different secretory organelles: rhoptries, which discharge at the time of cell invasion, and dense granules, which exocytose into the vacuole space once invasion is complete. The rhoptry proteins ROP2 and ROP4 are exposed on the cytoplasmic face of the PVM, and may mediate interaction with host cell organelles. The dense granule protein GRA3 inserts into the PVM in association with a change in conformation and polymerization state, and may mediate pore formation. We suggest that parasite proteins associated with the PVM play an important role in the intracellular survival of *T. gondii*.

B 006 ESCAPE FROM A VACUOLE AND CELL-TO-CELL SPREAD OF *LISTERIA MONOCYTOGENES*, Daniel A. Portnoy. Department of Microbiology, University of Pennslyvania School of Medicine, Philadelphia PA

Listeria monocytogenes is a gram positive facultative intracellular bacterial pathogen. There is an excellent murine model of infection where immunity is entirely cell-mediated. In addition, excellent models of infection have been developed using tissue culture cells. It is now clear, that subsequent to internalization, *L. monocytogenes* escapes from the host vacuole and grows rapidly in the cytoplasm of the infected cell. Soon after, the bacteria nucleate the polymerization of host actin filaments to mediate movement of the bacteria first to the periphery of the cell and then from cell-to-cell. A number of bacterial determinants of pathogenicity have been described of which two (listeriolysin O and ActA) will be discussed in this presentation.

Listeriolysin O (LLO), is a secreted pore-forming hemolysin which is necessary for the escape of *L. monocytogenes* from a host cell vacuole. LLO is a member of a large family of hemolysins which include among others streptolysin O and perfringolysin O (PFO). PFO has the same specific activity as LLO, although it functions identically at both pH 7.4 and 5.6. In contrast LLO has a pH optimum of 5.6. We replaced the structural gene encoding LLO with that encoding PFO in *L. monocytogenes*. The resulting strain was able to escape from the hsot vacuole, grow in the cytoplasm, in the absence of gentamicin and spread cell-to-cell. However, in the presence of high concentrations of gentamicin, the intracellular bacteria were killed. Somewhat surprisingly, the PFO-expressing strain was almost as avirulent as a LLO minus strain. The results suggest that one of the unique properties of LLO is that it is able to mediate lysis of the host cell vacuole without damaging the cell. In contrast, PFO is able to mediate escape from the vacuole, but causes the host cell to become permeable.

Once in the cytoplasm, ActA is the major surface protein made by *L. monocytogenes*. Mutants with in-frame deletions in *actA* are 3-logs less virulent and are absolutely defective for nucleation of actin filaments and hence cell-to-cell spread. Whereas ActA isolated from extracellular grown bacteria migrates as 97-kDa protein upon SDS-PAGE, ActA from intracellular bacteria migrates as a triplet of approximately 97-kDa, 100-kDa and 103-kDa. The higher molecular mass species were due to phosphorylation of the ActA protein. The significance of the phosphorylation of ActA is not yet known.

B 007 MOLECULAR AND CELLULAR ANALYSIS OF INTESTINAL BARRIER INVASION BY *Shigella flexneri*, Philippe J. Sansonetti, Unité de Pathogénie Microbienne Moléculaire, INSERM U 199, Institut Pasteur, 28 rue du Docteur Roux, F-75724 PARIS Cédex 15, FRANCE.

Shigellosis, or bacillary dysentery, is an invasive disease of the human colonic epithelium which is caused by a Gram-negative bacterial pathogen : *Shigella*. Molecular and cellular bases of the invasive process by *Shigella flexneri* can be studied at two levels : *in vitro* and *in vivo*. *In vitro*, bacterial and host-cell factors have been identified which allow efficient colonization of an epithelial monolayer : *Shigella* invasins (IpaB, C and D) allow entry into epithelial cells *via* bacterium-directed phagocytosis, the same invasins then achieve lysis of the phagocytic vacuole. IcsA, an ATPase expressed at one pole of the bacterium then allows intracellular movement and cell-to-cell spread through polymerization of cell actin ; bacterial protrusions are then transported from cel to cell by a process involving cell-adhesion molecules. Bacteria then lyse the double membrane of the protrusion and newly infected cell, reach the cytoplasm of this cell and so on. This cycle of infection represents a highly efficient mean of colonization. *In vivo*, a much more complex profile of this infection process emerges. Bacteria are unable to enter the epithelium *via* the apical surface of the cell. A strong inflammatory reaction with immigration of polymorphonuclear cells in the intestinal barrier is characteristic of shigellosis. It disrupts the integrity of the epithelial layer, and allows enterocyte infection and killing. We have demonstrated that after infection by *S. flexneri*, resident macrophages of the lymphoid follicles associated with the intestinal mucosa overcome a process of cell programmed death during which IL1 is released at high level. This is most probably the trigger that elicits the inflammatory reaction which, paradoxically, may represent the primary and not the secondary step in shigellosis.

B 008 INFLUENCE OF SURFACE CHARGE ON BIOLOGICAL BEHAVIOR OF *NEISSERIA GONORRHOEAE*, John Swanson, NIAID Laboratory of Microbial Structure & Function, Rocky Mountain Laboratories, Hamilton, MT 59840

Neisseria gonorrhoeae presents several different molecular species on its surface to interface with the external millieu. These include pili, multiple outer membrane components, and "loosely-associated" polyphosphate. Individual and collective contributions of these diverse molecules to the overall properties of *N. gonorrhoeae* are regularly reflected by changes in colonial morphology; but the physicochemical basis for such changes are poorly understood. Because *N. gonorrhoeae* surface components play key roles in the biological and pathogenetic behaviors of this organism, I have been studying the affect(s) that assorted surface components [pili, lipooligosaccharide (LOS), sialylation of LOS, colony opacity-associated proteins (Opa), and polyphosphate production] have on electrophoretic mobilities (EPM) of whole, intact bacterial cells. All influence EPM. Cells expressing different combinations of LOS, Opa, and pili can exhibit strikingly disparate EPMs; and some display apparent positive surface charge. LOS with short saccharide side chains imparts higher negativity than LOS with longer side chains. Sialylation of LOS markedly enhances cell negativity. The affects of particular Opa on EPM correlate with the charged amino acid constitutions of their predicted exposed regions; but such affects are highly dependent on presence of external polyanions (heparin, DNA). The modified charge due to some but not all Opa proteins is obscured by the negativity conferred by LOS sialylation. Pili in general engender marked reduction in negativity of cells, but the magnitude of this varies depending on which pilin subunit comprises the pilus polymers. Correlation between the adhesive behavior of particular *N. gonorrhoeae* variants toward tissue culture cells and their EPM suggest that surface charge is involved; but this can depend on whether the cells accrete a "garbage polyanion capsule" from their external millieu.

Molecular Interactions Between Viruses and Host Cell Membranes

B 009 REOVIRUS PATHOGENESIS - ROLE OF THE M2 GENE, Bernard N. Fields[1] and Max L. Nibert[2], [1]Harvard Medical School, Boston, MA 02115, [2]University of Wisconsin, Madison, WI 43706.

Reovirus outer capsid proteins play distinct roles in virus-host and virus-cell interactions. The σ1 protein (S1 gene) is the cell attachment protein and is involved in tropism and spread in the host. The μ1 protein (M2 gene) interacts with cell membranes and plays a role in neurovirulence. In this talk I will discuss how the early steps of entry of reovirus into the GI tract of a young mouse lead to conversion of the intact particle to an intermediate subviral particle (ISVP). The ISVP contains an extended σ1 protein with the μ1 protein (and its cleaved fragments) exposed on the capsid surface.

We have recently developed assays to analyze reovirus-membrane interactions. These assays are: a) chromium release to assay membrane injury and b) development of ion channels in lipid bilayers. Biochemical analysis of the μ1 protein has revealed features likely to be involved in membrane interaction. The μ1 protein is myristoylated at its N terminus. In addition, it is cleaved during treatments with exogenous proteases that generate ISVPs. This cleavage generates a large N terminal fragment (δ) and a small c-terminal fragment (ϕ). Both fragments stay on the ISVP. The δ:ϕ cleavage junction is flanked with sequences suggesting long amphipathic α helices.

The correlations between μ1 structure and the functions of μ1 in virus-cell and virus-host interactions provide a useful model for understanding a "molecular basis of viral pathogenesis."

B 010 WHAT TRIGGERS THE UNCOATING OF INCOMING VIRUSES? Ari Helenius, Urs Greber, Ila Singh, Matt Bui and Gary Whittaker, Department of Cell Biology, Yale School of Medicine, New Haven, CT 06510

When viruses enter cells, they have to be dismantled to release the genome and accessory proteins in a replication-competent form. These, moreover, have to be transported to the correct site of replication which may be the nucleus or specific locations within the cytoplasm. The mechanisms involved in uncoating, nuclear transport and targeting of incoming animal viruses are poorly understood at present.

We have analyzed these early events for three different viruses: Semliki Forest virus, Influenza virus A, and Adenovirus 2. Of these, adenovirus is a nonenveloped virus while the others are enveloped. For all three, the uncoating occurs in a stepwise fashion triggered by specific signals, and mediated by cellular factors inadvertantly supplied by the cell. In the case of adenovirus, uncoating starts already on the plasma membrane (or immediately after endocytic uptake) by the release of the fibers. This is followed by the dissociation and/or degradation of proteins IIIa, VIII and VI, and the loss of the penton base protein. Some of these proteins are thought to link the viral DNA to the inside surface of the capsid, while the penton base has been suggested to serve as an acid-activated lysis factor. In the cytosol further structural proteins are either degraded or dissociated from the virion as it proceeds to the nuclear pore complex. Final disassembly occurs at the nuclear membrane with the release of DNA through the nuclear pore into the nucleoplasm. The signals that determine the stepwise dismantling apparently include surface receptor attachment, low pH, exposure to a reducing environment, and attachment to a nuclear membrane receptor.

In the case of influenza virus, we think that the uncoating begins in the early endosomes by the entry of the protons through M2 channels present in the viral membrane. The acidification of the interior of the virus causes some yet unidentified conformational changes which allows the vRNPs and other components including the M1 protein to dissociate from each other once the virus has undergone its low pH-dependent membrane fusion reaction in late endosomes. The vRNPs are transported into the nucleus by active transport through nuclear pores.

Semliki Forest virus uses yet another mechanism. The nucleocapsids dissociate immediately after they are delivered to the cytosol by an acid activated membrane fusion event which takes place in early endosomes. The vRNA is released from its isometric shell. The capsid proteins that make up this shell are bound to high affinity sites present on the 28S RNA of the 60S ribosomal subunits. Thus, ribosomes serve as uncoating factors for this virus.

Exploitation of Host Cell Organelles in Early Infection

B 011 HOST CELL CYTOSKELETON REMODELING: LESSONS LEARNED FROM *LISTERIA*, Frederick S. Southwick[1], Jean M. Sanger[2], Katherine L. Phaneuf[1], Daniel L. Purich[1], Joseph W. Sanger[2], [1]University of Florida College of Medicine, Gainesville, FL 32610 & [2] University of Pennsylvania School of Medicine, Philadelphia, PA, 19104

Listeria monocytogenes is capable of moving through the cytoplasm of host cells at rapid rates. This process is driven by host cell actin polymerization. An actin filament structure forms behind the bacterium as it moves. This actin rocket tail remains fixed in the cytoplasm, progressively lengthening as the bacterium migrates forward (Dabiri, Sanger, Portnoy, Southwick, *PNAS*.87:6608,1990). Actin monomers are incorporated at the bacterial-actin tail interface while depolymerization takes place throughout the tail at a constant rate (Sanger, Sanger, & Southwick, *Infect. & Immun.* 60:3609,1992). These findings predict that actin rocket tail length should directly correlate with bacterial speed and actin tail elongation rate, *i.e.* the faster the speed of migration the longer the rocket tail (Theriot et al. *Nature* 357:257, 1992). To study the requirements for host cell actin monomers, 3 different monomer-sequestering proteins were microinjected into host cells infected with the 10403S wild type strain of *Listeria*. Introduction of profilin at final concentrations of 1-3 μM had little effect on *Listeria* movement or bacterial induced actin assembly, while high concentrations (20-30 μM) blocked intracellular movement and actin rocket tail assembly. Microinjection of profilin also slowed the disassembly rate of the bacteria-associated actin tails, filament half-life increasing from 1.2 to >30 min. Lower concentrations of Vitamin D binding protein (1-2 μM) and DNase I (6-7 μM) had similar effects to profilin. To study the effects of the actin capping and severing protein gelsolin on *Listeria*-induced actin assembly, clones of 3T3 fibroblasts permanently transfected with cytoplasmic gelsolin cDNA which overexpress intracellular gelsolin (Cunningham *et al.* Science 251:1233,1991) were used. Three cell lines C8, C4, and C5 contained, respectively, 1.25 x, 1.9 x and 2.25 x the relative intracellular gelsolin concentration of native 3T3 cells. These cells were fixed 4-6 hours after initiation of *Listeria* infection and stained with rhodamine-conjugated phallacidin, followed by anti-Listeriolysin 0 and a fluorescein-conjugated secondary antibody. Using dual fluorescence image analysis the lengths of individual actin rocket tails were measured in each cell line. Actin tail length was linearly related to intracellular gelsolin content (r=0.93). Mean tail lengths were 6.9 ± 0.3 μm (SE, N=52) in C8 cells, 8.4 ± 0.5 μm (N=58) in C4 cells and 9.5 ± 0.5 μm (N=91) in C5 cells. The differences in actin rocket tail length between C8 and C4 as well as between C8 and C5 were statistically significant (p=0.01 and p<.0001 respectively). The speed of intracellular bacterial migration was also measured using time-lapse video microscopy. *Listeria* migrated at significantly higher speeds in C5 cells as compared to control fibroblasts (p< .0001). The mean speed of intracellular migration in C5 cells was 0.13 ± 0.006 μm/sec (SE, N=119) as compared to 0.08 ± .004 μm/sec (SE, N=83) in control cells. The peak speeds attained by *Listeria* in C5 cells (0.24-0.36 μm/sec) were much higher than in control cells (0.11-0.18 μm/sec). Ligand binding studies are presently underway to better define the nature of the interactions of these important contractile proteins. We conclude that free actin monomers are required for *Listeria* motility and that gelsolin enhances the rate of *Listeria*-induced actin assembly and the rate of intracellular movement. These studies support the supposition that profilin and gelsolin play key roles in actin-based motility and point to the utility of the *Listeria* model for understanding how nonmuscle cells remodel their cytoskeleton during chemotaxis and phagocytosis.

Strategies Used by Microorganisms to Cross Epithelial Barriers

B 012 ENTERIC PATHOGEN INTERACTIONS WITH EPITHELIAL CELLS, B. Brett Finlay, Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., CANADA.

Salmonella typhimurium **and enteropathogenic *Escherichia coli* (EPEC) are two enteric pathogens which interact intimately with host epithelial cells. However, they do so by completely distinct mechanisms, ultimately achieving different end results. For example, both pathogens send different signals to the host cell, resulting in different rearrangements in the host cytoskeleton. Both increase the permeability of polarized monolayers, but by different means. Additionally, *S. typhimurium* is an efficient invader of epithelial cells. This organism is contained in a vacuoloar structure inside epithelial cells which has a different microenvironment than that found extracellularly. Finally, *S. typhimurium* is able to create a novel organelle that appears linked to intracellular replication. The interactions between these two organisms with cultured epithelial cells will be compared and contrasted.**

B 013 SALMONELLA-HOST CELL INTERACTIONS WHICH LEAD TO MEMBRANE RUFFLING AND BACTERIAL ENTRY, Bradley D. Jones[1], Nafisa Ghori[1], and Stanley Falkow[1,2], [1]Stanford University, Stanford, [2]Rocky Mountain Laboratories, NIH, Hamilton.

Virulent *Salmonella typhimurium* strains initiate systemic infection of mice by penetrating the intestinal epithelium, primarily at the ileal Peyer's patches (1,2). Our laboratory has published work demonstrating that the ability of *S. typhimurium* to penetrate the membrane of mammalian cells is induced by growth in a low oxygen environment. As a result, we have searched for *Salmonella* invasion genes, using *lac* gene fusions, which are expressed in low oxygen conditions. This screen has yielded a noninvasive, oxygen-regulated *lac* fusion mutant, BJ66. Here we report the results of an electronmicroscopic study comparing the ability of invasive *S. typhimurium* SL1344 and the ability of the noninvasive mutant BJ66 to interact with murine Peyer's patch tissue in a ligated loop model. Host cell changes induced by *Salmonella* could be observed by electronmicroscopy within thirty minutes of inoculation of invasive bacteria into a closed murine intestine. Invasive *Salmonella* were found to preferentially interact with and enter the specialized epithelial M cells of the Peyer's patches. In every instance, the bacteria had induced a dramatic rearrangement of the apical surface of the M cell as part of the mechanism leading to uptake. These membrane structures appear identical to actin ruffles which are induced by invasive *S. typhimurium* on the surface of polarized tissue culture cell monolayers. In addition, we observed that the presence of internalized bacteria had a cytotoxic effect on M cells as evidenced by mitochondria which were enlarged and had lost structural integrity. At sixty minutes post-infection, infected M cells were observed extruding into the lumen of the bowel. Complete destruction of the M cells created a point of entry which allowed extensive bacterial penetration of the gut epithelium. Following a 120 minute incubation of invasive *Salmonella* within the ligated loop, it was apparent that the organisms had free access to the lamina propia of the epithelium. At this timepoint we also often saw sections of epithelial cells sloughing away from the intact epithelium. In addition, bacteria were found within cells immediately beneath the epithelial layer of cells. Presumably, these intracellular bacteria would be carried by circulating cells into the lymphatic system from where they could establish a systemic infection. In contrast to invasive *S. typhimurium*, our efforts to infect murine intestinal tissue with the noninvasive mutant BJ66 were unsuccessful, even when 10-fold more bacteria were injected into the ligated loop. These results lead us to conclude that the *Salmonella* invasive machinery allows these bacteria to preferentially invade and destroy the M cells of Peyer's patches. The destruction of the M cells creates an opening through the intestinal epithelium which gives these pathogens free access to the lymphatic system of the host.

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Inflammation: Cellular and Extracellular Inducers - Methods of Intervention

B 014 CAMPYLOBACTER FETUS GENE REARRANGEMENT AND PERSISTENCE. Martin J. Blaser[1,2], Joel Dworkin[1], Enze Wang[1], Murali K.R. Tummuru[1], [1]Departments of Medicine and Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, [2] VA Medical Center, Nashville, TN.

Campylobacter fetus is a chronic colonizer of gastrointestinal and genital tract mucosa of ungulates, and may cause infertility and infectious abortion in a subset of infected hosts. Wild-type *C. fetus* cells possess regular paracrystalline surface layers composed of high molecular weight (97, 127, or 149 kDa) proteins, and these S-layer proteins (SLPs) are critical for serum and phagocyte resistance and virulence in a mouse model. These organisms also are able to vary the SLP expressed, which results in antigenic variation. *C. fetus* cells possess multiple homologs of *sapA*, encoding full-length SLPs, that contain both conserved and diverse regions. SLP variation is related to rearrangement of *sapA* homologs, a phenomenon that occurs at high frequency. Ability to rapidly create novel SLPs may be critical for persistence of *C. fetus* in its favored niches.

B 015 TRACHEAL CYTOTOXIN INDUCTION OF NITRIC OXIDE SYNTHESIS: ROLE IN THE PATHOGENESIS OF PERTUSSIS William E. Goldman, Kathryn E. Luker, Tod A. Flak, and Linda N. Heiss, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110.

The most striking cytopathological feature of *Bordetella pertussis* infection is the widespread destruction of ciliated cells in the respiratory epithelium. Because these cells are crucial for the normal clearance of secreted mucus, the resulting airway blockages lead to coughing episodes that are the most distinctive and disabling clinical feature of whooping cough (pertussis). Tracheal cytotoxin (TCT) was originally identified and characterized as the molecule that could reproduce this pattern of damage to ciliated cells in respiratory tract tissue from hamsters and humans. Despite having the destructive capacity and cellular specificity associated with an exotoxin, TCT bears no structural resemblance to conventional bacterial toxins. Instead, TCT is derived entirely from bacterial peptidoglycan, which *B. pertussis* processes and releases in the form of a 921 dalton disaccharide-tetrapeptide.

How can such a seemingly innocuous molecule generate the primary pathology of pertussis? We propose that TCT acts by initiating a self-destructive cascade of responses within the respiratory epithelium. The basis for this hypothesis is that TCT triggers the production of intracellular interleukin-1 (IL-1) by respiratory epithelial cells. Supporting a central role for this cytokine is the observation that recombinant IL-1, when added exogenously to respiratory tissue, completely duplicates the cytopathology of TCT. Although there are a variety of mechanisms by which IL-1 may affect mammalian cell function, our experiments suggest that IL-1 induction of nitric oxide synthesis is the pathway responsible for pertussis cytopathology. TCT or IL-1 stimulates nitric oxide production in respiratory epithelial cells, and we have demonstrated that this nitric oxide inactivates enzymes with iron-sulfur centers. Selective inhibitors of nitric oxide synthase abrogate TCT-induced production of nitric oxide and also block the pathological responses to TCT. An important co-factor in this pathway is endotoxin, which synergizes with TCT to produce much greater levels of IL-1 and nitric oxide than TCT alone. In organ cultures of respiratory epithelium, this effect is a significant acceleration of the usual time course of TCT pathology. The low concentrations of endotoxin required for this synergy can easily be accounted for by lipooligosaccharide release during *B. pertussis* growth. We have also evaluated structure-activity relationships for TCT damage to respiratory epithelium, and the structural features important for this cytopathology correlate precisely with those that are required to generate toxic levels of nitric oxide.

Our conclusion is that TCT, with the cooperation of endotoxin, triggers intracellular IL-1 production that in turn induces nitric oxide synthesis in respiratory epithelial cells. The specific cytopathology of pertussis is therefore an autotoxic response to nitric oxide, which inactivates enzymes that are critical for ciliated cell function and viability.

B 016 BACTERIAL COMPONENTS ACTIVE AT THE BLOOD-BRAIN BARRIER IN PROMOTING AND INHIBITING INFLAMMATION, Elaine Tuomanen, Rockefeller University, New York City.

Inflammation rapidly intensifies early in the antibiotic therapy of bacterial meningitis and down modulation of this response dramatically improves the outcome of disease in animals and humans. This implies that (1) soluble bacterial components incite the host response and (2) the host response is a participant in the process of blood brain barrier injury. Thus, the identification of these bioactive components and their interactions with host defenses is of significant medical importance. Endotoxin is accepted as a significant contributor to inflammation in gram negative disease but clearly does not act alone. Other bacterial components responsible for inflammation consist of a library of peptidoglycan fragments (muropeptides) which individually differ in structure and biological activity, thereby encoding for parts of the symptom complex of meningitis. Their combined presence summates to abnormal cerebrospinal fluid chemistry, enhanced blood brain barrier permeability, cerebral edema and increased intracranial pressure. These activities depend on structural features of the muropeptides which are different from their recognized structure/activity relationships for immunomodulation, ciliated cell cytotoxicity and induction of sleep. These components are released from both gram positive and gram negative bacteria and are equal to or more potent inflammatory agents than endotoxin. The receptors on the cerebral capillary endothelium for these muropeptides are currently being characterized and appear to participate in bacterial adherence to cerebral capillary endothelium, triggering of cytokine release and procoagulant activity on cerebral endothelium, and permeabilizing the blood brain barrier.

Based on an understanding of the pathophysiology of meningitis, the concept has arisen of adding an adjunct to antibiotic therapy to down modulate the host response to noxious components released during bacterial death. The critical endpoint appears to be elimination of leukocyte transmigration during the first few antibiotic doses. A number of modulators of leukocyte trafficking in the systemic circulation have been shown to have anti-inflammatory activity in the central nervous system. Of the most potent are components of pertussis toxin and filamentous hemagglutinin of *B. pertussis* which are capable of interfering with selectin-dependent and integrin-dependent leukocyte recruitment to brain respectively. Characterization of these prokaryotic examples of eukaryotic adhesion molecules has lead to identification of an endothelial adhesion receptor apparently unique to brain which functions in leukocyte trafficking and blood brain barrier permeability. Anti-bacterial antibodies cross reactive with the receptor or bacterial peptides mimicking the receptor can be engineered to induce a variety of therapeutically desirable activities, i.e. enhancement of blood brain barrier permeability in order to promote delivery of chemotherapeutic agents to brain or inhibition of leukocyte-derived injury to the brain during bacterial meningitis.

Developmental Changes that Occur in Vivo

B 017 SENSORY TRANSDUCTION AND THE CONTROL OF *BORDETELLA* VIRULENCE, M. Andrew Uhl, Brian J. Akerley, Peggy A. Cotter, and Jeff F. Miller, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

Genes and operons that encode bacterial virulence factors are usually subject to coordinate regulation in response to environmental signals. We are using *Bordetella pertussis* and *Bordetella bronchiseptica* as model systems to understand how bacterial genes that mediate the host-microbe interaction are regulated in *vitro* and in *vivo*. These closely related organisms cause whooping cough in humans and respiratory infections in lower animals, respectively. a. Molecular Mechanism of Signal Transduction. BvgS, a transmembrane sensor protein and BvgA, a transcriptional activator, act together to control expression of a large network of adhesins, toxins, and other factors required for pathogenesis. BvgA bears sequence similarity to regulator components, while BvgS shows similarity to both sensor and regulator components of bacterial two-component signal transduction systems. We have purified BvgA and the cytoplasmic portion of BvgS and have shown that BvgS is an autophosphorylating kinase that modulates the activity of BvgA through phosphorylation. A molecular-genetic approach was used to identify domains in BvgS that are involved in signaling. Functional consequences of signal transduction mutations were assessed by examining Bvg mediated gene expression in *vivo*, and by characterizing biochemical activities (autophosphorylation, phosphoryl group transfer, and multimerization) of purified proteins in *vitro*. Results show that BvgS autophosphorylates at a conserved histidine residue in its transmitter domain then transfers the phosphate to a conserved aspartic acid residue in its receiver domain. This event is prerequisite for the final step in which a phosphoryl group is directly transferred from phosphorylated BvgS to BvgA, allowing subsequent control of virulence gene expression. b. Genetic Organization of the *Bordetella* Virulence Regulon. In addition to positive control of adhesin and toxin production, we've recently found that the BvgAS system mediates negative regulation of motility and flagellar synthesis in *B.bronchiseptica*. Chemotaxis and motility are important for the establishment of many bacterial infections, and this observation demonstrates that the *Bordetella* virulence regulon is biphasic. We have cloned the flagellin structural gene, demonstrated transcriptional regulation by BvgAS, and identified several regulatory factors that couple the Bvg control system to negative regulation of the flagellin promoter. Our current studies are focused on an analysis of the regulatory circuitry involved in negative control of virulence gene expression in *B.bronchiseptica* and *B.pertussis*, and on the identification of BvgAS repressed factors with potential roles in infection and protection against disease. c. In *Vivo* Studies of Pathogenesis. *B.pertussis* has exclusively adapted to the human host, and available animal models are not appropriate for asking subtle questions about gene regulation and sensory transduction during infection and disease. For this reason we have established a rabbit model for *B.bronchiseptica* infection that mimics natural Bordetellosis. *B.bronchiseptica*, which causes bronchopneumonia, tracheobronchitis, and asymptomatic infections in lower animals, is closely related to *B.pertussis* and encodes a nearly identical BvgAS control system. This model provides us with an opportunity to analyze colonization, pathogenesis, the immune response, and the role of sensory transduction in the context of a natural host-parasite interaction. We have used information from *in vitro* studies to construct phase locked *Bordetella* strains with altered signaling characteristics and have assessed their ability to colonize and induce histopathology in the rabbit respiratory tract.

B 018 *SALMONELLA* COORDINATE GENE REGULATION WITHIN MACROPHAGE PHAGOSOMES. Samuel I. Miller, Infectious Disease Unit, Massachusetts General Hospital, Boston, MA 02114 (tel: 617-726-3818, fax: 617-726-7416)

The PhoP regulon, comprised of the genes regulated by the PhoQ (sensor-kinase) and PhoP (transcriptional-regulator) proteins, is necessary for *Salmonella typhimurium* mouse virulence, survival within macrophages, and growth on succinate as a sole carbon source. Loci termed *phoP* activated genes require PhoP/PhoQ for transcriptional activation. These loci are widely spaced on the chromosome and have been estimated to encode approximately 20 proteins, including a nonspecific periplasmic acid phosphatase and an 18 kD outer membrane protein with similarity to a number of proteins of diverse phenotypes, including a*Yersinia enterocolitica* epithelial cell invasion protein (Ail), a bacteriophage lambda outer membrane protein (Lom), a serum resistance protein (Rck) of *Salmonella typhimurium* , and a *Enterobacter cloacae* protein (OmpX) whose expression alters porin transcription and antibiotic sensitivity. Several other loci that require PhoP/PhoQ for expression have been defined as transcriptional and translational gene fusions formed by transposons. *pag* transcription is enhanced 70-100 fold 4 hours after *Salmonella* phagocytosis by macrophages but not epithelial cells. Transcriptional activation requires phagosome acidification below pH 5.0. Mutations in the periplasmic and presumed sensor domain of PhoQ that alter signal transduction have been defined. Single amino acid changes in this periplasmic domain can significantly reduce or activate *pag* expression. Morphologic evaluation of *Salmonella* interactions with macrophages indicate that organisms enter macrophages by macropinocytosis and reside within spacious phagosomes, membrane bound organelles that are similar to macropinosomes formed by growth factors and transforming agents. PhoP activated gene expression is correlated with the eventual shrinkage and acidification of these spacious phagosomes. Virulence defective *Salmonella* with constitutive activation of *pag* as a result of a periplasmic PhoQ mutation fail to induce macropinocytosis and spacious phagosome formation. These strains synthesize decreased amounts of a number of proteins encoded by *phoP* repressed genes. The defined *phoP* repressed loci encode envelope proteins; one locus *prgH*, is essential for *Salmonella typhimurium* mediated endocytosis by epithelial cells. We hypothesize that PhoP/PhoQ controls two phases of *Salmonella* gene expression mediated by contact with eukaryotic cells: early events involve the expression of *phoP* repressed genes and later intracellular macrophage signals modulate the expression of PhoP activated genes to promote intracellular survival .

Export and Cell Surface Localization of Virulence Factors

B 019 SECRETION OF Yop PROTEINS, Guy R.Cornelis, Abdel Allaoui, Marie-Paule Sory, Pierre Wattiau and Sophie Woestyn, Microbial Pathogenesis Unit, University of Louvain Medical School, Brussels, Belgium.

The pathogenicity panoply of Yersiniae consists of some surface structures and the secreted Yops. The Yops are secreted by an original machinery now considered as the archetype of type III secretion pathways. It does not involve the cleavage of a classical N-terminal signal sequence but the address is nevertheless localized in the N-terminal domain. There is no similarity between the exportation domains of the various Yops, indicating that the signal is essentially conformational. The system exports hybrid proteins formed by fusing the N-terminal domain of YopE or YopH and various prokaryotic or even eukaryotic proteins.
The system is encoded by the pYV plasmid. The distinction between regulatory genes and genes involved in secretion could be done on two criteria. First, one should find small amounts of Yops in secretion mutants but not in regulatory mutants. Second, mutations in the Yop secretion pathway should prevent the export of the Yops but not of YadA which is exported via the classical Sec pathway. The *virC* and the *virA* mutants fulfill these two criteria. The 8.5-kb *virC* locus constitutes a single operon composed of 13 genes called *yscA* to *yscM*. YscD and YscL were previously shown to be required for secretion. The analysis of a battery of non-polar mutants demonstrated that, at least YscF, YscI, YscJ and YscK are also required for the traffic of the Yops while YscH and YscM seems to be dispensible.
None of the *virC* products appears to be an ATP-binding protein. The *virB* locus encodes YscP, a 45.6-kDa protein containing a putative ATPase domain. The deletion of the Walker box A by site-directed mutagenesis completely abolished Yop secretion, which indicates first that this protein is indeed involved in the secretion pathway and, second, that the integrity of the ATPase domain is required for this action. YscP could act as the energizer of the system. More genes of the *virB* and *virA* loci could also be involved in secretion. Another likely candidate is *lcrD*, a gene of the *virA* locus.
One of the peculiarities of the Yop secretion system is that it makes use of cytoplasmic chaperones that are specific of individual Yops. We called these chaperones "Syc" for "specific Yop chaperone" followed by the code letter of the corresponding Yop. If a *syc* gene is mutated, the corresponding Yop is no longer exported but the secretion of the other Yops is not affected. The genes encoding *sycE* and *sycH* are localized next to the corresponding *yop* genes. We hypothesize that the role of these chaperones is to lead the nascent Yop proteins to the translocon. SycE is a 14.7-kDa protein with a very acidic (4.4) isoelectric point. It probably exists as a dimer in the cytoplasm. SycH is 16-kDa protein having also an acidic isoelectric point.
Secretion of some Yops is probably followed by their translocation into eukaryotic cells. To study this, we fused part of the *cyaA* gene of *Bordetella pertussis* to *yopE*. The hybrid protein was secreted, enzymatically active and effectively translocated into Hela cells as demonstrated by the increased intracellular cAMP concentration. The recombinant strain was not virulent for mice.

B 020 DETERMINANTS OF EXTRACELLULAR LOCALIZATION OF *PSEUDOMONAS AERUGINOSA* VIRULENCE DETERMINANTS, Stephen Lory, University of Washington, Seattle.

The opportunistic pathogen *Pseudomonas aeruginosa* elaborates a number of surface-associated and extracellular proteins which play an important role in the colonization of the human mucosal tissues. The attachment of *P. aeruginosa* to the epithelial cell receptors is mediated, in part, by type IV pili, which are composed of polymerized subunits of a single polypeptide, pilin. The assembly of type IV pilins on the bacterial surface requires two consecutive post-translational modifications carried out by a bifunctional enzyme, PilD, which cleaves and N-methylates the pilin subunit precursors. The subsequent assembly of the pilus organelle is mediated by several additional proteins, one of which is very likely an ATPase. Secretion of a number of exotoxins is directed by a specialized export machinery which shares several similarities with the pilus biogenesis apparatus. The machinery of extracellular secretion includes four proteins with a high level of similarity to the type IV pilins. The secretory apparatus therefore shows a requirement for functional PilD, for N-terminal processing and methylation of the four pilin homologues. The protein secretor apparatus also requires a functional ATPase sharing homology with the similar protein required for pilus biogenesis. Mutants of *P. aeruginosa* unable to export proteins and assemble pili are avirulent in animal models of infection, indicating that surface localization of virulence factors is an essential pathogenic trait of this microorganism. Recent work from several laboratories has shown that machinery of pilus biogenesis and extracellular protein secretion similar to that found in *P. aeruginosa* is present in a wide range of human, animal, and plant pathogens. The ability of pathogenic bacteria to effectively localize virulence determinants to their surface or to the surrounding media therefore necessitated evolution of a specialized export machinery, in addition to the machinery responsible for normal protein secretion.

B 021 TRANSLOCATION OF PROTEINS ACROSS MEMBRANES; Sanford M. Simon, Lab of Cellular Biophysics, Rockefeller University, 1230 York Avenue, New York, N.Y. 10021

Biological membranes are barriers to free diffusion. We have examined how the permeability of these barriers is modified to allow the selective transport of macromolecules. It has been known that ions are allowed to cross membranes through the opening of selective transmembrane channels. We will present data consistent with the existence of transmembrane channels for the transport of proteins across membranes.

Large aqueous channels were observed in membranes which can translocate proteins such as the plasma membrane of Escherichia coli and pancreatic endoplasmic reticulum-derived rough microsomes. There was a signficant increase in the number of channels upon release of nascent translocating polypeptides from the membrane. These channels could be reopened by the addition of synthetic signal peptides. These results indicate that the signal sequence is a sufficient ligand for opening these protein-conducting channels.

Recent experiments suggest that these channels may be a general mechanism for moving macromolecules such nucleotides, amino acids, and perhaps DNA and phage, across most cellular membranes. We will discuss their effects on cell physiology and their critical role in multidrug resistance.

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Late Abstract

PROTEOLYTIC ACTIVATION OF THE COMPLEMENT CASCADE, Tony E. Hugli, Richard G. DiScipio, Marleen Kawahara and James Travis* Dept. of Immunol, Scripps Research Institute, La Jolla, CA. 92037 *Dept of Biochem, U. of GA, Athens, GA 30602

Components C3 and C5 are pivotal proteins of the blood complement cascade. Their activation by limited proteolysis is central to the physiologic role of complement in host defense mechanisms. The smaller fragments C3a and C5a (called anaphylatoxins) are 9-11 kDa factors that promote chemotaxis, leukocyte and monocyte metabolic activation, spasmogenesis and exhibit immunoregulatory functions while the larger fragments C3b and C5b enhance phagocytosis and initiate cytolytic events. C3a and C5a participate in proinflammatory activities and when generated in excess can be pathobiologic. Possible examples are acute infections accompanied by a progressive inflammatory state such as adult periodontitis.The condition of advanced gingival tissue injury is promoted by anaerobic bacteria and their enzymatic products.The arginine-specific cysteine proteinase from *Porphyromonas gingivalis* (called Gingipain-1) cleaves both C3 and C5 with high specificity. A functional anaphylatoxin (i.e. C5a) is generated only from C5. Cleavage of human C3 by Gingipain-1 is selective and scission occurs at or near the C3a/C3b scission site on the C3 α chain; however C3a activity is not detected in the digest. We concluded that the C3a (9 kDa and 77 residues) is rapidly degraded by the proteinase once it is released. The primary cleavage site for Gingipain-1 in C5 occurs between residues 715-716 of the α-chain and a secondary cleavage occurs more slowly between residues 74-75 releasing the C5a. Since the C5a molecule appears to be more resistance to Gingipain-1 than does C3a, it survives digestion and is demonstrated functionally as an intact factor. A similar series of experiments have been carried out using a lysine-specific cysteine proteinase from *P. ginivalis* and this proteinase cleaves C3, but not at the C3a/C3b site.The cleavage pattern indicates that C3 α chain is degraded extensively while the more resistant β chain is partially degraded. An intial cleavage probably occurs near the mid-portion of the α chain generating two fragments of approximately 60 kDa. One of these fragments is further degraded while the other appears to be resistant. C5 is also digested by lysine-Gingipain. We will discuss implications of proteinases produced in the anaerobic microbiota of the infected gingiva and their effects on plasma components capable of releasing potent inflammatory factors at the site of injury.

Surface Molecules on Pathogens Responsible for Invasion and Disease: Attachment and Entry; Intracellular Interactions Between Pathogens and Host

B 100 MECHANISMS OF BINDING OF *BRUCELLA ABORTUS* TO MACROPHAGES OF CATTLE GENETICALLY RESISTANT TO BRUCELLOSIS, L. Garry Adams, Gregory A. Campbell, Joe W. Templeton and Blair A. Sowa, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467

Brucella abortus is a Gram negative facultative intracellular bacterium which causes abortion in cattle and undulant fever in man. Macrophages are the target host cells of B. abortus and cell mediated immunity is thought to be primarily responsible for acquired resistance against brucellosis. Specific inhibition of B. abortus adherence to peripheral blood monocyte derived macrophages of genetically resistant and susceptible cattle was studied. Macrophages phagocytosed live B. abortus without opsonization. Phagocytosis was inhibited form the resistant cattle by the peptide RGDS, outer membrane-peptidoglycan complex from B. abortus, anti-LFA-1 monoclonal antibody, anti-C3 antiserum, fibronectin, purified O-antigen from B. abortus lipopolysaccharide, mannan, and heat-inactivated bovine IgG. Phagocytosis of both resistant and susceptible cattle was inhibited by outer membrane-peptidoglycan complex, anti-LFA-1, O-antigen from B. abortus lipopolysaccharide and heat-inactivated bovine IgG. The RGES peptide did not inhibit phagocytosis by macrophages from resistant or susceptible cattle, thus, indicating the importance of the RGD peptide in functioning as a receptor for the binding and phagocytosis of B. abortus in resistant cattle. Phagocytosis in both resistant and susceptible cattle appears to be mediated by binding to LFA-1 and a cell wall component and/or O-antigen. In susceptible cattle, phagocytosis in the presence of several inhibitors implies an additional or augmented mechanism of uptake, which may be related to the pathogenesis of brucellosis in cattle. The O-LPS and the RGD peptide sequence located in the OMP of *B. abortus* were shown to be of major importance in binding and phagocytosis of B. abortus in cattle.

B 101 IDENTIFICATION OF A TWO-COMPONENT REGULATORY SYSTEM IN *STAPHYLOCOCCUS AUREUS* THAT CONTROLS THE EXPRESSION OF SURFACE COMPONENTS. Kenneth W. Bayles, UMBC, Baltimore, MD 21228

The sensor proteins of bacterial two-component regulatory systems are histidine kinases that comprise a large family of proteins involved in environmental sensing and signal transduction. To study sensor proteins from *Staphylococcus aureus*, degenerate oligonucleotides that correspond to conserved DNA sequences contained within sensor genes were synthesized. Using these oligonucleotide primers, DNA fragments from *S. aureus* were amplified by PCR, cloned in *Escherichia coli*, and sequenced. A comparison of the deduced amino acid sequences from these cloned fragments to the sequences in GenBank revealed that some had similarities to sensor proteins and, surprisingly, others had similarities to ATP-dependent permeases (many permeases have been shown to be histidine kinases, possessing environmental sensing and signal transducing ability). Because of its strong homology to sensor proteins, one PCR clone (designated *kin1*) was selected for further analysis. Cloning and sequencing of the entire *kin1* gene and flanking DNA revealed that *kin1* potentially encodes a 65 Kd protein that contains all of the conserved regions found in sensor proteins. Furthermore, immediately adjacent to *kin1* was another open reading frame (designated *reg1*) that potentially encodes a protein with 27% amino acid sequence identity to *Pseudomonas aeruginosa* AlgR, a known response regulator that controls capsule production in this organism. To determine the function of *kin1*, a null-mutation of *kin1* was made by Campbell integration. This mutant (termed KIN1) exhibited a dramatic propensity to clump when grown in liquid culture, suggesting that the expression of surface components in this strain was modified. The molecular nature of this phenotype and the role (if any) of *kin1* and *reg1* in the regulation of capsule production in *S. aureus* are currently under investigation.

B 102 *MYCOBACTERIUM AVIUM* COMPLEX BINDS TO AND INVADES HT-29 AND HEp-2 EPITHELIAL CELLS, Luiz E. Bermudez and Kathleen Shelton, Kuzell Institute for Arthritis and Infectious Diseases of the California Pacific Medical Center Research Institute, San Francisco, CA 94115

Mycobacterium avium is associated with bacteremia and disseminated infection in patients with AIDS. Current evidence indicates that prior to causing infection, *M. avium* must colonize and invade the intestinal mucosa. This study examined the ability of *M. avium* complex to bind and invade HT-29 intestinal mucosal cell line and HEp-2 pharyngeal cell line. *M. avium* was more efficient in binding to and invading when cultured to logarithmic phase of growth than when culture to stationary phase of growth. Binding and invasion was also influenced by the temperature of growth. Bacteria incubated at 37° and 40° C was shown to adhere and invade HT-29 cells more efficiently than bacteria cultured at 30° C. The ability of *M. avium* to invade HT-29 and HEp-2 cells was inhibited when epithelial cells were incubated with cytochalasin B prior to exposure to the bacterium, suggesting active participation of the mammalian cell in the process of internalization. Incubation of eukaryotic cells with Protein Kinase inhibitors (staurosporin and H7) inhibited the internalization of the bacterium. Tyrosine Kinase inhibitor (Genistein) also inhibits invasion suggesting that bacterial uptake may depend on the phosphorylation of proteins on epithelial cell membrane. *M. avium* DNA was cloned in the plasmid pMV306 and inserted in *E. coli* K12. The transformed *E. coli* were able to invade HEp-2 cells and HT-29 cells. A sequence homology between *M. avium* DNA and the *inv* gene of *Yersinia* was found and cloned into pMV306 plasmid. This locus confers *E. coli* with the ability to invade HEp-2 epithelial cells. These findings suggest that *M. avium* binds to specific receptor(s) on the epithelial cells and uses the cytoskeleton of the mammalian cell to be internalized. Furthermore, *M. avium* DNA shares a sequence homology with *Yersinia inv* gene that is associated with the ability to invade epithelial cells.

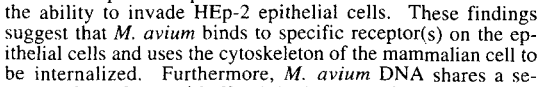
B 103 **STRUCTURAL CHARACTERIZATION AND IMMUNOCHEMISTRY OF THE CAPSULAR ANTIGEN OF *Streptococcus pneumoniae* SEROTYPE 35B,** Linda M. Beynon, J. C. Richards, and M. B. Perry, Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada, K1A 0R6.

Despite the availability of antibiotics and a multivalent capsular polysaccharide vaccine, diseases caused by *Streptococcus pneumoniae* remain some of the most prevalent and serious infections in the world especially in young children and the elderly.

Capsular antigens are responsible both for the stimulation of the immune response and for the virulence of encapsulated bacteria. Although invasiveness depends on both the size and composition of the capsule, the latter is more important.

There are 83 serologically defined pneumococcal types based on their capsular polysaccharide. The Danish typing scheme combines these into closely related groups. Structural analysis of the capsular polysaccharides has led to an exact definition of the epitopes involved in their specificities and identified minor structural features such as O-acetylation which result in the definition of sub-serotypes such as the serotypes 11A, 11B, 11C, and 11F in serogroup 11.

The capsular antigen of *S. pneumoniae* serotype 35B was analysed by a combination of 2D NMR methods, mass spectrometry and classical carbohydrate chemistry. The polysaccharide was found to be a high molecular weight polymer composed of a repeating unit containing D-galactose, D-glucose, 2-acetamido-2-deoxy-D-galactose, and ribitol. The pentasaccharide is polymerized through phosphate diester linkages.



B 104 EVIDENCE FOR M PROTEIN IN EQUINE UTERINE ISOLATES *OF STREPTOCOCCUS ZOOEPIDEMICUS*, Robert C. Causey, William J. Todd, Dale L. Paccamonti, Department of Veterinary Microbiology and Parasitology, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803

We conducted studies to determine if strains of *Streptococcus zooepidemicus* isolated from the equine uterus possess an antiphagocytic M-like protein. In immunoblots of twelve phage lysin digested isolates, monoclonal antibody to the conserved region of type 6 M protein bound two isolates, as did anti M 6 polyclonal serum, which also bound a third isolate. Polyclonal serum directed at a cloned M-like protein of *Streptococcus zooepidemicus* was broadly reactive across twelve isolates, but recognized the same proteins as the anti M 6 antibodies. Seven of eighteen isolates grew in fresh blood of one horse (horse A), the remainder being killed. Of these seven, one was killed in the blood of a second horse (horse B). Killing was directed by plasma of horse B, but required white cells from either horse. Heat treated plasma of horse B could also mediate killing, suggesting phagocytosis mediated by antibody, but not complement. One isolate which grew in blood of horses A and B was killed by blood of the horse from which it was isolated, also suggestive of antibody directed phagocytosis. Although limited in scope, to date these data support the hypothesis that some equine uterine isolates of *Streptococcus zooepidemicus* carry a type specific M-like protein which confers resistance to phagocytosis unless type specific antibody is present.

B 105 DIFFERENCES IN GLYCOSAMINOGLYCAN (GAG)-MEDIATED ATTACHMENT TO HOST CELLS BY TRACHOMA AND LGV BIOVARS OF *CHLAMYDIA TRACHOMATIS*, Joseph C.-R. Chen and Richard S. Stephens, Department of Laboratory Medicine and the Francis I. Proctor Foundation, University of California, San Francisco, CA 94143-0412

Chlamydia trachomatis is an important human pathogen that is subdivided into two biovars—trachoma and LGV. Although trachoma and LGV biovars share near identity by genetic and phylogenetic criteria, they cause significantly different clinical syndromes. It has recently been reported that a GAG-dependent mechanism is required for chlamydial attachment and entry into mammalian host cells. Since virulence differences between the biovars may also be reflected in their behavior in tissue culture systems, we examined whether the two biovars share the same mechanism for interaction with eukaryotic cells. Although exogenous heparin inhibits infectivity of both biovars and inhibits LGV biovar attachment to host cells, we found that the trachoma biovar attachment was only modestly inhibited by exogenous heparin. Using a specific heparan sulfate lyase (heparitinase) to treat organisms, the attachment of LGV biovar was abolished. In contrast, the attachment of trachoma biovar treated with a four times higher concentration of heparitinase was reduced only by about 50%. Significantly, attachment could be fully restored for heparitinase-treated organisms of both biovars with exogenous heparan sulfate. Thus, these data suggest that there are quantitative and/or qualitative differences in the heparan sulfate-like ligand for the two biovars, nevertheless they share the same fundamental mechanism for infection of host cells.

B 106 A *CRYPTOSPORIDIUM PARVUM* MULTIDRUG RESISTANCE GENE, Douglas P. Clark, Tu D. Tu, and Douglas Jones, Department of Pathology and Laboratory Medicine, The University of Pennsylvania, Philadelphia, PA 19104.

Cryptosporidium parvum (Apicomplexa: Cryptosporidiidae) is an obligate intracellular protozoan parasite which infects the intestinal tract of mammals. In human AIDS patients, *C. parvum* causes a severe diarrheal illness resulting in dehydration, malabsorption, and even death. Despite testing of over 100 different drugs, no drug therapy is effective against *C. parvum*. One possible explanation for this drug resistance is the expression of a multidrug resistance (mdr) protein, similar to the human mdr molecule implicated in the resistance of tumor cells to chemotherapeutic drugs, or the mdr gene amplified in drug-resistant strains of *Plasmodium falciparum*.

Using primers to nucleotide sequences conserved among known mdr genes, we have amplified a 283 bp fragment of a *C. parvum* mdr gene from *C. parvum* genomic DNA using the polymerase chain reaction. This DNA fragment appears to encode a nucleotide binding domain, based on its homology to other mdr molecules. Specifically, the deduced amino acid sequence of this fragment is nearly 50% identical to the first nucleotide binding domain of several mammalian mdr molecules including human p-glycoprotein 1, Chinese hamster p-glycoproteins 1 and 2, and mouse p-glycoproteins 1 and 3. The *C. parvum* mdr fragment also shows considerable similarity to known protozoan mdr molecules including *Plasmodium falciparum* mdr 2, a *Leishmania enriettii* mdr protein, and a *Leishmania donovani* mdr protein.

Future studies will include characterization of the entire *C. parvum* mdr gene, identification of additional *C. parvum* mdr molecule family members, and establishment of their role in C. parvum drug resistance.

B 107 PROTEIN DEPENDENT PEPTIDE PERMEASES FROM *STREPTOCOCCUS PNEUMONIAE* MEDIATE CYTO-ADHERENCE TO TYPE II LUNG CELLS AND TO HUMAN ENDOTHELIAL CELLS. Diana R. Cundell, Barbara J. Pearce, Alison Young, Elaine I. Tuomanen and H. Robert Masure. Laboratory of Molecular Infectious Diseases, The Rockefeller University, New York, NY 10021

To create a profile of pneumococcal infection of the lower respiratory tract, pneumococcal adherence to cells isolated from rabbit lung was assessed in vitro. Isogenic encapsulated and unencapsulated bacteria adhered equally well with the preferred target being the Type II alveolar cell. To investigate this interaction, a cyto-adherence assay to a human cultured type II alveolar cell line (T2LC) was developed and contrasted to human vascular endothelial cells (HUVEC). Fluorescein-labelled pneumococci were allowed to adhere to the two cell types for 30 min at 37°C. The cells were then washed, fixed and adherent bacteria were enumerated using an inverted microscope. Live and heat killed (60°C, 10 min) unencapsulated pneumococci bound in a similar, dose dependent manner to both cell types. The presence of a type 2 capsule did not alter this binding.

We have recently developed a technique to genetically identify and mutate exported proteins in pneumococcus based on gene fusions to alkaline phosphatase (*phoA*) [B. J. Pearce *et al*, *Mol. Microbiol.* **9**, 1037-1050, 1993]. To identify bacterial elements involved in adherence, we screened a bank of independent $PhoA^+$ mutants with defects in exported proteins for their ability to adhere to T2LCs and HUVECs. Two mutants demonstrated a >60% decrease in adherence to both cell types in six independent assays. Sequence analysis of the recovered loci showed that each encoded a distinct protein-dependent peptide permease: *amiA* and *plpA*: (permease like protein). The derived nucleotide sequence of PlpA was 60% identical and 80% similar to AmiA. We conclude that peptide permeases modulate adherence of pneumococci to epithelial and endothelial cells, either by acting directly as adhesins or, more likely, by regulating the presentation of adhesins on the pneumococcal surface.

B 108 *Salmonella typhimurium* IS TARGETED TO LYSOSOMES WITHIN EPITHELIAL CELLS BYPASSING VACUOLES CONTAINING MANNOSE-6-PHOSPHATE RECEPTOR. Francisco Garcia-del Portillo and B. Brett Finlay. Biotechnology Laboratory and the Departments of Microbiology and Biochemistry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Salmonella typhimurium is an intracellular pathogen which invades and replicates within non-phagocytic cells. This bacterium is targeted to lysosomal membrane glycoprotein (lgp)-containing vacuoles after invading cultured human epithelial cells. In the present study we have characterized the interaction of *S. typhimurium* with other host cell organelles during targeting to lysosomes of HeLa epithelial cells. Our results show that intracellular bacteria are not colocalized at any post-infection time with vacuoles containing the mannose-6-phosphate receptor (M6P-R), which supplies soluble lysosomal enzymes to lysosomes. Vacuoles containing M6P-R are considered an intermediate stage in lysosome biogenesis. Additional results, obtained using antibodies against an enzyme that traffics to lysosomes without requiring transport via M6P-R, such as human lysosomal acid-phosphatase (LAP), confirmed that LAP is present in *S. typhimurium*-containing vacuoles, suggesting that although vacuoles with intracellular bacteria do not fuse with M6P-R-containing vacuoles, they have lysosomal enzymes. We are currently in the process of determining whether other lysosomal enzymes, specially those depending on M6P-R for transport to lysosomes, are present in the vacuoles containing *S. typhimurium*, thus defining the biogenesis of vacuoles containing this intracellular bacterium.

B 109 REGULATION OF SURFACE PRESENTATION OF IcsA, A *Shigella flexneri* PROTEIN ESSENTIAL TO INTRACELLULAR MOVEMENT, IS CELL CYCLE DEPENDENT. Marcia B. Goldberg[1] and Philippe J. Sansonetti[2], Albert Einstein College of Medicine, Bronx, NY 10461[1], and Pasteur Institut, Paris[2].

Shigella flexneri is a gram-negative bacterial pathogen that invades intestinal mucosal cells and spreads from cell-to-cell, thereby causing diarrhea and mucosal abscesses. Following entry into the host cell, the bacterium lyses the phagocytic vacuole. Then, within the cytoplasm it may begin to accumulate short filaments of polymerized cellular actin on its surface at one pole. In conjunction with bacterial movement, through the cytoplasm these actin filaments form a tight bundle, called an actin tail, that extends behind the bacterium from that pole. The genetic locus *icsA (virG)* has been previously identified as essential to intracellular movement and cell-to-cell spread (*Proc Natl Acad Sci USA* 86:3867, 1989). Not every bacterium that is observed within the cytoplasm of an infected cell will have an actin tail attached to it. Many bacteria are without actin tails. The factors that determine when a bacterium will move are unknown.

We have recently characterized a secreted Shigella surface protein, IcsA, that interacts with the actin tail (*J Bacteriol* 175:2189, 1993). On the surface of the bacterium, IcsA is a 120-kDa protein that is located at a single pole, and on dividing bacteria, it is evident that the pole that contains IcsA is the distal pole, thereby necessarily placing it adjacent to the growing end of the actin tail.

We have previously observed that at the moment of initiation of movement within the cytoplasm, a bacterium is frequently in the process of division. Moreover, 85% of bacteria observed at the tips of protrusions are in the process of division (Prevost et al, 1992). We were therefore interested in examining the association of bacterial division with the initiation of movement. In this study, we demonstrate that the expression of IcsA on the surface of the bacteria occurs in a cell-cycle dependent fashion. The percentage of bacteria surface-labelled with IcsA antibody increased throughout the early and late exponential phases of growth from 13% to 87%, with a subsequent fall to 28%. The relative intensity of labelling changed in parallel. These data suggest that the surface expression of IcsA *per se* determines the observed association of bacterial division with movement.

B 110 SELECTIVE LABELING OF PROTEINS SYNTHESIZED BY INTRACELLULAR PARASITES USING RICIN AND HOST CELLS LACKING MITOCHONDRIAL DNA. Anne M Gurnett[1], Paula M Dulski[1], Dennis M Schmatz[1] and Mark Carrington [2], (1) Merck Research Laboratories, Dept. Parasite Biochemistry and Molecular Biology, PO Box 2000, Rahway, NJ, 07065, (2) University of Cambridge, Dept of Biochemistry, Tennis Court Rd, Cambridge, England.

Studies focused on the synthesis of developmentally regulated proteins by intracellular parasites have been limited due to the lack of a simple method for selectively labeling proteins produced by the parasite. A method has now been developed in which the toxin, ricin, is employed to selectively inhibit host cell protein synthesis while protein synthesis by the intracellular parasite is unaffected. Ricin is composed of two subunits, one of which binds to cell surface receptors containing terminal galactose residues while the other subunit enters the cell, inactivates ribosomes and, as a consequence, cytoplasmic protein synthesis. Due to the loss of the receptor binding subunit ricin cannot permeate the host cell mitochondria or the intracellular parasite, and therefore protein synthesis within these compartments continues uninterrupted. This system has been explored using *Eimeria tenella* infected avian rho zero cells. This host cell type was selected because it lacks mitochondrial DNA and supports the intracellular development of *E. tenella* sporozoites through first generation merogony. Host mitochondrial proteins are not synthesized when labeling in the presence of ricin because these cells lack mitochondrial DNA. Therefore, those proteins which are radiolabelled with ^{35}S methionine in ricin treated infected monolayers are exclusively those of the intracellular parasite. Alternatively cells with intact mitochondria can be utilized, here the host mitochondrial protein synthesis can be inhibited by chloramphenicol.

A time course study was conducted to identify those proteins which are produced throughout the parasites intracellular phase and those which exclusively appear during specific stages of development. Identification of these proteins will lead to a better understanding of intracellular parasite development and provide a means to select stage specific antigens for further study. This technique is applicable to the study of protein synthesis in other intracellular parasites.

B 111 MODULATION OF GONOCOCCAL RESISTANCE TO TOXIC HYDROPHOBIC ANTIMICROBIAL AGENTS BY THE MEMBRANE PROTEIN MtrC. Kayla E. Hagman,[1] Wubin Pan,[2] Brian G. Spratt[2] and William M. Shafer,[1] [1]Department of Microbiology and Immunology, Emory University School of Medicine and VA Medical Center, Atlanta, GA, USA, [2]School of Biological Sciences, University of Sussex, Falmer, Brighton, UK.

Neisseria gonorrhoeae, in contrast to other Gram-negative organisms, exhibits higher permeability to toxic hydrophobic agents such as fatty acids that bathe certain mucosal sites. Consequently, viability of gonococci is inhibited in the presence of these compounds. Infection of the human host at sites with high concentrations of hydrophobic agents, such as the rectum, has been suggested to select for organisms with increased resistance to toxic fatty acids and other hydrophobic agents. One mutation, *mtr* (multiple transferrable resistance), imparts nonspecific, low-level resistance to hydrophobic drugs, dyes and detergents in the gonococcus and also increases expression of a 44 kDa outer-membrane protein, MtrC. The nonspecific, hydrophobic agent resistance phenotype of Mtr strains appears to be dependent on the presence and level of MtrC, a membrane lipoprotein. To evaluate the role of MtrC in determining resistance to toxic hydrophobic agents we sought to clone, sequence and characterize the *mtrC* gene. Loss of expression of the MtrC protein due to insertional inactivation of the *mtrC* structural gene causes increased sensitivity to these agents compared to wild-type, parental strain FA19. We now report the cloning and characterization of *mtrC*.

B 112 THE AMINO-TERMINAL DOMAIN OF THE P-PILUS ADHESIN DETERMINES RECEPTOR SPECIFICITY

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The *PapG* protein, located at the distal tip of P-pili, mediates adherence of P-fimbriated *E. coli* to the globoseries glycolipids. The amino-terminal portion of the *PapG* adhesin demonstrates considerable sequence heterogeneity between human and canine isolates. These variations define three classes of adhesins, which display minor differences in receptor specificity.

We constructed fusion proteins containing the *E. coli* maltose binding protein (MBP) fused to the amino-terminal 195 amino acids from each of the three adhesin classes. Here we report the binding pattern of the fusion proteins to globoseries glycolipids following HPTLC overlays. The fusion protein derived from the class I adhesin binds globotriosylceramide (GbO3) and globoside (GbO4) most avidly, and demonstrates minimal adherence to Forssman antigen (GbO5). In contrast, the class III fusion protein demonstrates intense adherence to GbO5 and negligible binding to GbO3 and GbO4. These results are identical to binding experiments utilizing intact bacteria and pili purified from each of the three classes. ELISA assays utilizing serial dilutions of globoseries glycolipids confirm the variation in receptor specificity. In contrast to the N-terminal fusions, a protein containing the carboxy-terminal 135 amino acids of the class I adhesin fused to the MBP demonstrated negligible adherence to all glycolipids examined. A monoclonal antibody (MAb) raised against the class III fusion protein was specific for class III pili and was found to inhibit adherence of the class III fusion protein to immobilized GbO5. Preincubation of intact bacteria expressing class III adhesin with this MAb markedly reduced adherence of the organism to MDCK II cells.

These data confirm that variations in the N-terminus of the *PapG* adhesin are responsible for fine variation in receptor specificity, and thereby determine host and tissue tropism. Further analysis of these proteins should allow detailed mapping of residues involved in the carbohydrate-binding domain. Moreover, their stimulation of antibodies which inhibit adherence of intact bacteria to host cells suggests they may be ideal vaccines. We have exploited the ease of constructing and purifying MBP fusion proteins; an approach that may have wide applicability to the study of bacterial adhesins and the production of vaccines against bacterial components.

B 113 INTERACTION OF *PORPHYROMONAS GINGIVALIS* AND *CAMPYLOBACTER RECTUS* WITH HEp-2 EPITHELIAL CELLS.

Stanley C. Holt, Wiebke Kennell, Beinan Wang, and Jeffrey L. Ebersole. U. of Texas Health Science Center at San Antonio, TX 78284.

A body of literature indicates that the adherence and ultimate invasion of host cells is an essential event in pathogenesis. The interaction with, and invasion of host cells by oral pathogens is problematic. Recent evidence from our laboratory indicates that both *P. gingivalis* (*Pg*) and *C. rectus* (*Cr*) adhere to the surface of epithelial cells of the HEp-2 cell line. *Pg* both adhered, and invaded these mammalian cells. Both bacterial strains were grown under standard conditions. The cells were harvested, and resuspended in an appropriate transport buffer. HEp-2 cells which were cultured to semi-confluency were exposed to the bacterial strains at MOI's between 10:1 and 10^4:1. Indirect immunofluorescence using anti-*Pg* or *Cr* antibody was used to determine the extent of interaction. SEM and TEM were used to reveal the temporal events in adherence and cell invasion. *Pg* strains W50 and 381 adhered to the HEp-2 surface at a level of approx. 10% of the initial inoculum. Strain 381 was at least 4-fold more adherent that W50. Live bacteria adhered better than dead cells. Growth of *Pg* under hemin-limiting conditions resulted in at least a 100-fold greater adherence of *Pg* than those grown under hemin normal conditions. This interaction was blocked by L-arginine and L-lysine. SEM observations revealed the bacteria to interact with the surface of the HEp-2 cells along its entire surface. TEM revealed an interaction between the *Pg* "capsule" and the HEp-2 plasma membrane. Temporally, the bacteria were located within the HEp-2 cytoplasm; both within vacuoles and "free". *Cr* adherence to HEp-2 cells appeared to be regulated by their S layer. In the presence of this outermost layer, *Cr* was essentially refractory to interaction with the HEp-2 cells; however, loss of the S layer by *in vitro* passage resulted in a significant increase in adherence. The adherence was also dependent upon *Cr* growth phase with stationary phase cells 3x less adherent than logarithmic phase cells. TEM revealed a direct interaction between the *Cr* outer membrane and the HEp-2 plasma membrane. **These observations suggest that *P. gingivalis* and *C. rectus* are capable of adhering to, and invading HEp-2 epithelial cells, and that this host cell interaction and invasion might represent one mechanism by which these oral pathogens destroy the integrity of the periodontium.**

B 114 *Abstract Withdrawn*

B 115 X-ADHESINS OF URO-PATHOGENIC *ESCHERICHIA COLI* BIND TO THE SCR-4 OF THE DECAY ACCELERATING FACTOR (DAF), EPITOPES PROXIMAL TO THE CELL MEMBRANE.

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Dr and related uropathogenic AFA-I, AFA-III and F-1845 diarrhoea associated adhesins recognize epitopes on Short concensus repeat-3 (SCR-3) of the DAF as demonstrated by the lack of interaction of recombinant fimbriae to SCR-3 deletion and other mutants of DAF. A large number of *E.coli* adhesins associated with urinary tract infections and diarrhoea, express adhesins of unknown receptor specificity and are called X-fimbriae. In this study, we attempted to characterize X-adhesins of gestational pyelonephritis associated *E.coli*.

Six *E.coli* isolates that express X-adhesins were tested for reactivity with Chinese Hamester Ovary (CHO) cells that express DAF or various DAF deletion mutants. Attachment was estimated following incubation of the *E.coli* isolates or purified fimbriae with CHO cells.

Attachment to a given DAF deletion mutant was calculated as the number of *E.coli* cells / per single CHO cell or by computer based image analysis system to measure the integrated optical density.

Total DNA was isolated from the given *E.coli* isolates and used for Polymerase chain reaction (PCR) with set of primers designed for *dra* related sequences. All the six tested isolates contained *dra* related fragments as all of these showed 750bp DNA amplification product.

Attachment analysis of the PCR positive isolates showed binding to all DAF-positive and DAF deletion mutants except to SCR-4 deletion or in some isolates DAF-HLA B44, where the anchoring GPI sequences were replaced by a non-related transmembrane molecule.

The above data is consistent with the hypothesis that number of X-adhesins may belong to the family of DAF recognizing adhesins. It remains to be investigated whether this interaction is associated with activation of the complement as DAF function is to control the complement cascade at the C3 level.

B 116 MAPPING AN ADHESION EPITOPE WITHIN THE CELL SURFACE ANTIGEN OF *STREPTOCOCCUS MUTANS*. C.G.Kelly, G.H.Munro, S.Todryk, H.Kendal, P.Evans, P.Buckett and T.Lehner, Department of Immunology, United Medical and Dental Schools of Guy's and St Thomas's Hospital, London SE1 9RT, U.K.

Attachment of *Streptococcus mutans* to the tooth surface is mediated by a cell surface protein of M_r 185,000 termed streptococcal antigen (SA) I/II. Sequencing of the gene showed that it encodes a protein of 1561 amino acid residues which includes a leader sequence (residues 1-38). The aim of this study was to identify regions of SA I/II involved in binding. Five overlapping gene portions were amplified by polymerase chain reaction, cloned and expressed in *Escherichia coli*. The recombinant polypeptides comprised residues 39-481 (fragment 1), 475-824 (fragment 2), 816-1213 (fragment 3), 816-1161 (a truncated form of fragment 3) and 1155-1538 (fragment 4). The recombinant polypeptides were assayed for adhesion binding activity to salivary receptors and for recognition by a panel of monoclonal antibodies (MAbs), raised against SA I/II. Two of the MAbs which are known to prevent colonisation of *S. mutans in vivo* bound the recombinant polypeptide comprising residues 816-1161. *In vitro* adhesion of *S. mutans* to saliva coated hydroxyapatite beads was also inhibited specifically by polypeptide fragment 3 encompassing the same region. Overlapping synthetic peptides (20ers) derived from the sequence of fragment 3 have been used to map the adhesion epitope more precisely. The evidence from the MAbs preventing colonisation of *S. mutans* and the adherence inhibition assay suggest that an adhesion-binding activity resides within the portion of SA I/II comprising residues 816-1213 which is highly conserved amongst oral streptococcal species.

B 117 INTERACTION OF AN *Aeromonas hydrophila* ISOLATE WITH CULTIVATED HUMAN EPITHELIAL CELLS. Dennis. J. Kopecko and Tobias A. Oelschlaeger, Dept. of Bacterial Diseases, WRAIR, Washington, DC 20307-5100. *Aeromonas hydrophila* is not only an important fish pathogen but also is suspected to be a probable cause of diarrhea in humans. Known virulence factors include α and β hemolysins and cytotonic enterotoxin. Additionally, internalization ability into HEp-2 cells has been demonstrated and may be one of several mechanisms by which *Aeromonas* strains exert their enteropathogenicity. In the present study the invasion ability of the *A. hydrophila* isolate 102, obtained from a monkey with diarrhea, was quantitated in invasion assays with different human epithelial cell lines using gentamicin to kill extracellular bacteria. Furthermore, by employing a variety of compounds in the invasion assays the role of de novo protein synthesis and the possible involvement of certain structures and processes of epithelial cells in the internalization of strain 102 was determined. The invasion efficiencies into cultivated epithelial cells of the bladder (T24, 5637), the ileum (INT407) or the ileocecum (HCT-8) reached 3% to 7% of the inoculum at the beginning of the assay. Bacterial de novo protein synthesis, but not eukaryotic de novo protein synthesis, was a prerequisite for *A. hydrophila* strain 102 to enter any of the cell lines studied. For efficient internalization into 3 of the 4 cell lines included in this study, microfilaments were essential. Additionally, uptake of strain 102 into all 4 cell lines was strongly inhibited by microtubule depolymerization. Moreover, stabilization of microtubules reduced entry to different degrees of *A. hydrophila* 102 into 3 cell lines. Inhibition of endosome acidification by monensin had only a severe effect on internalization into HCT-8 cells, whereas ammonium chloride, which also interferes with endosome acidification, reduced uptake into all 4 cell lines dramatically. Besides inhibiting invasion, ammonium chloride also drastically reduced adherence to the epithelial cells. These findings show the ability of *A. hydrophila* strain 102 to adhere to and invade different human epithelial cells by an uptake pathway(s) that generally rely on bacterial de novo protein synthesis, microfilaments, microtubules and endosome acidification.

B 118 SPECIFIC CHANGES IN THE SURFACE GLYCOPROTEIN OF INFLUENZA C VIRUS ARE ASSOCIATED WITH A LONG-TERM PERSISTENT PHENOTYPE

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A long-term persistence model has been established by selecting a spontanous mutant of influenza C/AA/1/50 virus in a permanent carrier culture of MDCK cells. Infectivity and cell tropism are mainly determined by the multifunctional viral membrane glycoprotein (HEF). HEF analysis was aimed at clarifying persistence-specific sequence variations and attributing these to functional properties, i.e. receptor binding, enzymatic activity, fusion and antigenic epitopes. The emerging picture is summarized by the following findings: (i) C/AA/1/50 persisting virus carries a modified receptor-binding sequence, (ii) the receptor-binding activity is altered as indicated by a higher efficiency in recognizing low amounts of the receptor determinant, (iii) direct attachment to cell surfaces differs from wild-type virus, as measured by slower kinetics in viral elution, (iv) receptor-destroying enzymatic activity is diminished, (v) recognition of persistent-type HEF epitopes by MAbs is affected and (vi) characteristic features of virion surface morphology seem to be altered, respectively instable. The sum of these changes highlights a structurally and functionally modified HEF glycoprotein which allows for long-term viral persistence. Analysis as to which of the described points constitutes a requirement for persistence is presently in progress.

B 119 A GENE HOMOLOGOUS TO THE EUKARYOTIC FKBP IMMUNOPHILIN FAMILY IS LINKED TO *pilS* OF *NEISSERIA MENINGITIDIS*. Carl F. McAllister and David S. Stephens. Emory University School of Medicine and VAMC, Atlanta, GA, USA.

Human FKBP is a recently discovered immunophilin gene which was cloned and expressed by Standaert et al. (Nature 346:671). FKBP has peptidyl-prolyl-*cis-trans*-isomerase activity and is thought to be an important regulator of signal transduction in a number of eukaryotic cells. Extensive DNA and amino acid homology was shown to exist between human FKBP and a region of DNA sequence upstream from the single *pilS* locus in two Class II piliated strains (C114 and FAM18) of *N. meningitidis*. We designed primers to amplify via PCR a 238 bp region encompassing 73% of the FKBP ORF. A PCR product was obtained for all 14 meningococcal strains screened, including Class I and Class II piliated strains of various capsular serogroups. One strain gave a 221 bp product due to a 17 bp deletion within FKBP. A 238 bp product was also obtained for all 12 commensal *Neisseria* spp. that we screened, but no product was obtained from any gonococcal strains (11 screened) or from *Moraxella catarrhalis*. We sequenced the PCR products for five meningococcal and seven commensal strains. The DNA and predicted amino acid homologies were ≥97% among the meningococcal strains and ≥93% and ≥97%, respectively, between the commensal and meningococcal strains. Southern hybridizations confirmed the absence of an FKBP gene in gonococcal strains and showed that a single copy of the FKBP gene was closely linked to a *pilS* gene in all meningococcal strains examined. We have identified a 13 kb meningococcal DNA insert containing the FKBP gene from a lambda library. Sequencing of this clone confirmed the presence of an upstream promoter and ribosome binding site and a *pilS* gene 22 bp downstream from the FKBP stop codon. There did not appear to be a transcriptional terminator between the two genes. A homologue of the eukaryotic FKBP gene is highly conserved in meningococci and is closely linked to *pilS*. FKBP homologues are also found in commensal *Neisseria* spp., but not in *N. gonorrhoeae*.

B 120 CLONING, MUTAGENESIS, AND VIRULENCE ANALYSES OF A FLAGELLIN GENE OF VIBRIO ANGUILLARUM, D.L.Milton, R.O'Toole, and H. Wolf-Watz, Department of Cell and Molecular Biology,University of Umeå S901-87 Umeå, Sweden.

Recently, the sheath of the flagellum was shown to be a virulence factor for the fish pathogen *Vibrio anguillarum*. This finding prompted the cloning, sequencing, and mutagenesis of the flagellin gene. The DNA sequence suggests that the *flaA* gene encodes a 41.8 kDa protein and is monocistronic. Southern analysis determined a single chromosomal copy. A 180-bp in-frame deletion of a part of the amino-terminus believed to be involved in the polymerization of the flagellin into the flagellum and a polar mutation, generated by plasmid integration within the *flaA* gene, were made. For both mutants, electron microscopy displayed a shortening of the flagellum. The flagellum was shown to consist of four major proteins with molecular masses of 43, 44, 45, and 46 kDa and the 43 kDa protein was shown to correspond to the *flaA* gene product. N-terminal amino acid sequence analysis indicated that the three remaining proteins were also flagellin proteins with N-termini that are 82-88% identical to the FlaA protein and to each other. Fish virulence studies showed that the deletion mutant gave a 100-fold increase in the LD_{50} with infection via immersion, whereas infection via intraperitoneal injection showed no loss in virulence. In contrast, the polar mutant showed a $3.5x10^4$-fold increase in LD_{50} for both immersion and intraperitoneal infection. Although transcomplemetation studies have not been completed, the two *flaA* mutations, which display two different virulence phenotypes, suggest that the flagellum may aid *V. anguillarum* in both entry into the fish and replication within the fish. What role the FlaA protein versus the additional flagellin proteins have in virulence as well as if the sheath acts in conjunction with the flagellin proteins in aiding virulence must still be discerned.

B 121 GENETIC DIVERSITY AND PATHOGENIC ROLES OF THE MAJOR CELL SURFACE GLYCOPROTEINS (MSG) OF *PNEUMOCYSTIS CARINII*

Yoshikazu Nakamura, Miki Wada, Hiroshi Yoshida and Kazuhiro Kitada, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan

Pneumocystis carinii is a eukaryotic microbe that often causes fatal pneumonia in AIDS patients. *P. carinii* has an unusually abundant and highly immunogenic surface glycoproteins called MSG (or P115/gp120) that plays a crucial role in the pathobiology of *P. carinii*. The MSG fractions are composed of several isoelectric variants and highly glycosylated with mannose. Deglycosylation analysis revealed that the sugar moiety constitutes the strong epitope of MSG. The cDNAs encoding MSG polypeptides were cloned from a λgt11 expression library of rat-derived *P. carinii* by immuno-screening using antibodies raised against deglycosylated MSG molecules. The cloned cDNAs constituted a MSG gene family containing 70% amino acid identity between subtypes. The diversity of MSG cDNAs was high and reflected the genomic structure of MSG genes clustered in most of the *P. carinii* chromosomes. These multiple genes may account for the high level expression of MSG that could generate potential variations in the cell surface to escape from host immunity. Interestingly, the MSG sequences have significant sequence homology to tropomyosins and myosins, suggesting physical or functional association with the membrane cytoskeleton. It is tempting to speculate that MSG may transmit an environmental signal across the outer membrane to the membrane cytoskeleton during proliferation and establishment of an extracellular infectious focus of *P. carinii* in immunocompromised states in man or experimental animals. The role of MSG in host cell attachment and pathogenesis will be presented.

B 122 ANALYSIS OF THE MEMBRANE AND SOLUBLE FORMS OF THE COMPLEMENT REGULATORY PROTEIN OF *Trypanosoma cruzi*, Karen A. Norris and Jane E. Schrimpf, Department of Molecular Genetics and Biochemsitry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

A developmentally regulated surface glycoprotein of *Trypanosoma cruzi* binds the third component of complement, restricting complement activation, thus providing the parasites a means of avoiding the lytic effects of complement. This complement regulatory protein, CRP, was shown to bind human C4b, a component of the classical pathway C3 convertase, and may therefore also act to restrict classical complement activation. The protein shares functional, biochemical and genetic similarities with the mammalian complement regulatory protein, decay accelerating factor. Characterization of carbohydrate modification of the protein revealed extensive N-linked glycosylation and no apparent O-linked sugars. CRP purified from parasites treated with an inhibitor of N-linked glycosylation exhibited a decreased binding affinity for C3b compared to the fully glycosylated protein. The protein is anchored in the membrane via a glycosyl phosphatidylinositol linkage and spontaneously shed from the parasite surface. Release of CRP was time and temperature dependent, and inhibited by zinc. The majority of the shed CRP had an apparent molecular mass of 160 kDa and lacked the glycolipid anchor, whereas the membrane form was recovered with the glycolipid anchor attached and had an apparent molecular mass of 185 kDa. Both the membrane form and soluble form retained binding affinity for C3b. Evidence is presented to suggest that the release of CRP from the parasite membrane is the result of cleavage by an endogenous phospholipase C.The release of CRP may augment the protection of the parasites from complement mediated lysis by the removal of complement-CRP complexes.

B 123 SOME CHARACTERISTICS OF EHEC INTERNALIZATION INTO HUMAN EPITHELIAL CELL LINES. Tobias A. Oelschlaeger and Dennis J. Kopecko, Dept. of Bacterial Diseases, WRAIR, Wahington, DC 20307-5100

Several enterohemorrhagic *Escherichia coli* isolates (O157:H7) were all found to be able to invade certain human epithelial cells in vitro. Their ability to gain entry into epithelial cells was compared to known invasive *Shigella flexneri* and *Salmonella typhi* strains in invasion assays utilizing gentamicin in order to kill extracellular bacteria. All EHEC strains efficiently invaded T24 bladder and HCT-8 ileocecal cells independent of the presence or absence of the sero type-specific 60MDa plasmid. In contrast to *S. flexneri*, these EHEC strains were not internalized into INT407 or HEp-2 cells. Although intracellular EHEC, as *Shigella*, were detected by electron microscopy to be free in the cytoplasm, in contrast to *Shigella*, EHEC were not observed to multiply in the cytoplasm of the host cell in the early hours postinfection. Furthermore, other than the invasion prerequisite of de novo bacterial (but not eukaryotic) protein synthesis the uptake pathways of EHEC versus *Shigella* and *Salmonella* seem to be quite different. Internalization of *S. flexneri* and *S. typhi* into human epithelial cells employed in this sudy was only inhibited by microfilament (MF) - depolymerization. In contrast, EHEC internalization into T24 cells was reduced by microtubule (MT)-depolymerization, MF-depolymerization and inhibition of coated pit formation. Uptake of EHEC into HCT-8 cells however, was reduced only by MF-depolymerization and interference with endosome acidification and not by MT-depolymerization or inhibition of coated pit formation. These findings demonstrate the ability of these EHEC strains to enter human epithelial cells by variant uptake pathways, which are different from the internalization mechanism of *S. flexneri*, although both organisms cause a bloody diarrhea.

B 124 **SIGNAL TRANSDUCTION BETWEEN GROUP A STREPTOCOCCI AND HUMAN PHARYNGEAL CELLS.** Vijaykumar Pancholi*, and Vincent A. Fischetti, Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, NY 10021.

While a great deal of information is currently available about the surface proteins of group A streptococci, little is known regarding their role during early events in infection of tonsillar/pharyngeal cells. We recently identified and characterized a novel major surface protein, which we termed streptococcal surface dehydrogenase (SDH) that exhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity and binds a variety of mammalian proteins. More recently, we found that in addition to its GAPDH activity, SDH is also an ADP-ribosylating enzyme, which, in the presence of NAD, results in the auto-ADP-ribosylation and inhibition of its GAPDH activity. SDH also has an ADP-ribosyl transferase activity, transferring the ADP-ribose moiety of NAD to free cysteine. Both the auto-ADP-ribosylating and ADP-ribosyl transferase activities were found to be significantly enhanced in the presence of nitric oxide (NO·). Since both ADP-ribosylation and NO· play a central role in signal transduction, we examined if SDH was in fact involved in such events. When the membranes of Detroit 562 human pharyngeal cells were interacted with SDH in the presence of [^{32}P]ATP, normal phosphorylation was found to be impaired, resulting in the predominent phosphorylation of 32 kDa-protein. This effect was not found when other human or animal cell lines were used and or when streptococcal M protein was substituted for the SDH. In a pulse chase experiment in the presence of SDH, the 32 kDa protein was found to be phosphorylated but unable to transfer the labeled phosphorus to other molecules, as seen when SDH was not present. These results strongly suggest that SDH plays a regulatory role in phosphorylation events which could have significant implications during the infection of pharyngeal cells.

B 125 THE USE OF AN INDUCIBLE PROMOTER FOR THE STUDY OF *M.tuberculosis* PATHOGENESIS. Tanya Parish, Philip Draper and Neil G. Stoker. Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, London, England, WC1E 7HT. Laboratory for Leprosy and Mycobacterial Research, National Institute for Medical Research, London, England, NW7 1AA.

The cell wall of *Mycobacterium tuberculosis* is a complex structure which has been implicated in the virulence of the organism, but little is known about the roles of individual molecules in pathogenesis. We wish to construct mutants of *M.tuberculosis* which lack individual genes, but to date it has not been possible to do this by standard gene replacement techniques, perhaps because of an inefficient homologous recombination system. One method to down-regulate gene expression without mutating the gene is by high level expression of the antisense strand.

We have been studying the promoter of the acetamidase gene of *Mycobacterium smegmatis*, a rapidly growing species. This enzyme enables the organism to grow on simple amides as the sole carbon sources, and is highly inducible in the presence of acetamide. A 4.1kbp genomic DNA fragment containing the amidase gene had previously been cloned. We have identified and sequenced regions required for inducible expression using a promoter probe vector containing a CAT (chloramphenicol acetyl transferase) reporter gene. We now intend to use the vector to express antisense mRNA of cell wall synthesis genes from *M.tuberculosis*. Such a system has the additional advantage of allowing the study of genes that are essential for growth, and which would not be obtainable as knockout mutants.

B 126 TRANSCRIPTIONAL REGULATION OF THE BUNDLE-FORMING PILIN GENE OF ENTEROPATHOGENIC *E. COLI*. Jose L. Puente, David Bieber, William J. Murray, Indira Sohel and Gary K. Schoolnik. Howard Hughes Medical Institute, Standford University Medical Center, Stanford University, Stanford, CA. 94305.

The initial stage of EPEC infection is characterized by the formation of bacterial microcolonies and their attachment to intestinal epithelial cells, a phenotype described as "localized adherence" (LA). LA requires the EAF plasmid that contains *bfpA* which codes for the subunit of the bundle-forming pilus (BFP).

The LA phenotype and the production of BFP on the bacterial surface are induced by growth in tissue culture media (e.g., DME) but not by growth in Luria broth (LB). Northern and western blot analysis show that in LB, *bfp*A is expressed and the BFP subunit is produced at low levels, however, pilus fibers are not detected by electron microscopy. In contrast, *bfpA* mRNA and the BFP subunit are strongly expressed in DME. In order to identify factors that might induce *bfpA* expression, EPEC strains B171 and B171/pBFP638, a pBR-derived plasmid containing the 638 bp, 5' upstream region of *bfpA* fused to the promoter-less CAT reporter gene, were propagated under different growth conditions. We found that differing growth kinetics result in variation in the maximal accumulation of both the *bfpA*-transcript and *bfp-cat* activity. Neither the *bfpA* transcript nor an increase in CAT activity were detected during the stationary growth phase. *bfpA* is expressed during the log phase of growth in all media but the levels of transcript accumulation depend on the duration of log phase growth. While LB sustains very rapid growth, the expression of *bfpA* occurs early (during the first 2-3 hours of growth) and is transient. In contrast, growth in DME results in the accumulation of the *bfpA* transcript at higher levels and for a longer time (5-6 hours).

Temperature, pH, osmolarity or anaerobiosis only affect *bfpA* expression to the extent that they also affect the growth rate. In addition to growth, *bfp*A expression is modulated by [Ca^{++}] and [NH_4^+]. B171/pBFP638 grown in DME lacking Ca^{++} demonstrated DME-like growth kinetics, but only produced 60% of the CAT activity observed when grown in DME with Ca^{++}. However, while the addition of NH_4^+ to DME did not affect growth, the CAT activity was significantly reduced.

These data suggest that BFP production is controlled at both a transcriptional and a translational level and that the expression of the BFP fiber is likely to be a function of growth phase and the concentration of calcium and ammonium.We are currently examining the role that the EAF plasmid plays in BFP regulation, since the expression of *bfp-cat* fusions is not induced in EAF-cured EPEC strains.

B 127 *ENTEROCOCCUS FAECIUM*-NEUTROPHIL INTERACTIONS: RESISTANCE TO PHAGOCYTOSIS BY A CARBOHYDRATE-MEDIATED MECHANISM, Robert M. Rakita, Roberto C. Arduino, Karen Jacques-Palaz, Barbara E. Murray, Center for Infectious Diseases, University of Texas, Houston, TX 77030

Enterococci are now the second most common cause of nosocomial infections. The incidence of their resistance to antimicrobials has risen dramatically, and isolates are now not infrequently resistant to all clinically useful antibiotics. A better understanding of enterococcal-host interactions could be extremely important in the development of alternative treatment strategies. In the course of characterizing the interactions of enterococci with human neutrophils (PMNs), we discovered two strains of *E.faecium* (DO and SM), isolated from patients with infective endocarditis, which were completely resistant to PMN-mediated killing in an assay which yielded >99% killing of other enterococci after 120 min exposure to PMNs. Phagocytosis was examined by labelling DO with 0.1% FITC in 50 mM carbonate buffer, pH 9.5 for 30 min, followed by opsonization with 10% serum, exposure to PMNs at 37°C for 30 min in a bacteria:PMN ratio of 10:1, and quenching of extracellular fluorescence with 50 μg/ml ethidium bromide. While control *E.faecalis* were phagocytosed appropriately, there was no phagocytosis or even association of DO with PMNs. This lack of association was confirmed by electron microscopy. Although many bacteria use capsules to inhibit phagocytosis, no capsule could be identified around DO by electron microscopy or with a modified Quellung reaction using specific rabbit antiserum. However, pretreatment of DO with 20 mM Na periodate for 20 min at 4°C followed by 10 mM $NaBH_4$ for 60 min at 25°C, (but not pretreatment with trypsin, pronase, or phospholipase C) eliminated the resistance of DO to phagocytosis. Sialic acid, a periodate-sensitive structure used by other Gram positive cocci, such as group B streptococci, to inhibit complement-mediated opsonization, could not be detected in DO using a thiobarbituric acid assay, and neuraminidase treatment did not alter DO's lack of association with PMNs. Thus, certain strains of *E.faecium* isolated from patients with endocarditis are resistant to phagocytosis by PMNs and a carbohydrate-containing moiety, which is not sialic acid, is likely to be responsible.

B 128 TRANSCRIPTIONAL ACTIVATION OF *SALMONELLA* VIRULENCE PLASMID GENES IN J774 CELLS,
Mikael Rhen, Pipsa Heiskanen, Petri Riikonen and Suvi Taira, Department of Biochemistry, University of Helsinki, PB 5, 00014 Helsinki, Finland

The intracellular growth phase of *S.dublin* and *S.typhimurium* in mice is dependent on a plasmid-carried *spv* gene cluster, which includes the transcriptional activator gene *spvR* and the *spvA, B, C* and *D* genes. This gene cluster is organized into two major transcriptional units, *spvR* and *spvABCD*, both of which respond to the activator SpvR. The transcriptional activity of the *spv* genes was studied using reporter operon fusions to *spvR* and *spvA* and by immunodetection of SpvR and SpvA. The *spv* genes appeared inactive in *S.typhimurium* grown in ordinary medium, whereas the *spv* operon fusions were activated in bacteria grown within murine macrophage-like J774 cells. The *spv* genes of *S.dublin* appeared active in culture medium and this activity was potentiated further when *S.dublin* was grown within J774 cells. The same phenomenon was reconstituted in *E.coli* grown in culture medium and harboring the virulence plasmid from either *S.dublin* or *S.typhimurium*; *spv* activity was detected only in the presence of the *S.dublin* virulence plasmid. The *spv* reporters were entirely inactive in *Salmonella* or *E.coli* strains cured of the virulence plasmid. We conclude that the intrinsic activities of the *spv* genes, or the *spvR* genes only, are different in *S.dublin* and *S.typhimurium* and that the *spv* gene clusters from both serovariants become activated in host cells.

B 129 MOLECULAR AND CELLULAR STUDIES OF ATTACHMENT AND INVASION OF *NEISSERIA MENINGITIDIS*, Efrain Ribot[1], Kristin Birkness[1], David Stephens[2] and Frederick Quinn[1], [1]Centers for Disease Control and Prevention, Atlanta, GA 30333 and [2]Emory University School of Medicine, Atlanta, GA 30322.

Neisseria meningitidis, a Gram-negative encapsulated diplococcus, is an obligate human pathogen. Although this organism has received a great deal of media and scientific attention in the past few years, little is known of the genetics and cell biology of the host-parasite interaction. Understanding attachment, invasion and passage through human epithelial and endothelial cells is one of the most important aspects in *N. meningitidis* disease. However, tissue culture assays for the study attachment and invasion has shed little light on the pathogenesis of the organism. Recent developments such as a transposon mutagenesis system and more relevant cell lines have aided the study of virulence of meningococcal meningitis. The purpose of this project is twofold: (1) to study the mechanism of infection of *N. meningitidis* at the cellular level and (2) identify the bacterial genes involved in this interaction. To accomplish this, mutants were created by the random insertion of transposon Tn916 into the *N. meningitidis* chromosome. Several hundred of the resulting mutants were selected at random and screened for an altered ability to attach/invade a human epithelial tissue culture cell line (HEC-1B) as compared to the parent strain (NMB). One mutant (VVV6) showed a significant decrease in its ability to attach and invade tissue culture cells. This mutant does not react with serogroup B type sera which may indicate an altered capsule. In addition, it has identical SDS-PAGE protein profile to its parental strain. The lower ability to attach and invade is likely linked to the disruption of a gene(s) possibly coding for a factor necessary for recognition and attachment to the host cell. We have detected the presence of the transposon in the mutant genome via Southern analysis and are using PCR and DNA sequencing analysis to identify the gene(s) involved. Identification of these gene(s) might shed some light in the mechanism of infection of this organism.

B 130 A RHOPTRY-SPECIFIC PROTEIN OF BABESIA BOVIS IS INDUCED BY OXIDATIVE AND NUTRITIONAL STRESS Allison C. Rice-Ficht[1], Shumin Zhao[2] and Wendy Brown[2], Departments of Medical Biochemistry and Genetics[1] and Department of Veterinary Pathobiology[2], Texas A & M University, College Station, TX 77843

Babesia bovis is a hemoparasite whose invasive process is rhoptry-mediated. We have examined a B. bovis specific protein of 77kDa which is localized to the rhoptry and is produced both during bovine infection and in microaerophilus culture. A cDNA encoding the protein has been isolated using hyperimmune serum and characterized (Tripp et al, Exp. Parasitol. 69, 211-225 (1989); this gene predominates in clonal isolation from an expression library when using either immune serum from natural infection or serum derived from immunization with parasite antigen. The gene encoding the 77kDa protein has been sequenced and is present in all B. bovis geographic isolates. A variation in protein yields in vitro spurred an investigation into the expression of this protein and revealed that it is over-expressed in response to oxidative or nutritional stress but not by heat shock. This suggests that it is regulated by promoters that respond to stress although the cDNA bears no sequence homology with the coding regions of any known stress protein genes. Because of their ubiquitous and highly conserved nature, stress proteins are believed to be functionally important and required for the successful transition a parasite makes from one host to another during its complicated life cycle. Antibody localization of the 77kDa gene product strongly suggests that the protein is stage-specific or developmentally regulated.

B 131 GENETICALLY ENGINEERED FMDVs INCAPABLE OF INFECTING CELLS VIA THE NATURAL RECEPTOR CAN DO SO BY AN ANTIBODY DEPENDENT ENHANCEMENT PATHWAY, Elizabeth Rieder, Barry Baxt, and Peter W. Mason, Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Greenport, NY 11944

Foot-and-mouth disease virus (FMDV) appears to initiate infection by binding to cells at an arginine-glycine-aspartic acid (RGD) sequence found in the flexible loop between beta strands G and H of the viral capsid protein VP1. The role of the RGD sequence in attachment of virus to cells was tested using synthetic full-length viral RNAs mutated within or near this three-amino-acid sequence. Baby hamster kidney (BHK) cells transfected with three different RNAs carrying mutations bordering the RGD sequence produced infectious viruses with wild-type plaque morphology; however, one of these mutant viruses bound to cells less efficiently than wild-type. BHK cells transfected with RNAs containing changes within the RGD sequence produced non-infectious particles indistinguishable from wild-type virus in terms of sedimentation coefficient, binding to monoclonal antibodies, and protein composition. These virus-like particles are defined as *rec*$^-$ viruses since they were unable to bind to and infect BHK cells. These mutant viruses were only defective in cell binding since antibody-complexed *rec*$^-$ viruses were able to infect Chinese hamster ovary cells expressing an immunoglobulin Fc receptor. These results confirm the essential role of the RGD sequence in binding of FMDV to susceptible cells, and demonstrate that the natural cellular receptor for the virus only serves to bind virus to the cell.

B 132 RECEPTOR FOR COXSACKIEVIRUS A9, Merja Roivainen[1], Liisa Piirainen[1], Tapani Hovi[1], Ismo Virtanen[2], Terhi Riikonen[3], Jyrki Heino[3], and Timo Hyypiä[3] National Public Health Institute, Helsinki, Finland, [2] Department of Anatomy, University of Helsinki, Helsinki, Finland, [3] University of Turku, Turku, Finland

The arginine-glycine-aspartic acid (RGD) motif close to the C-terminal end of the VP1 protein of coxsackivirus A9 (CAV-9) is needed for attachment of the virus to its cellular receptor as shown by a blocking test using RGD containing synthetic oligopeptides (M.Roivainen, T.Hyypiä, L.Piirainen, N.Kalkkinen, G.Stanway, T.Hovi, J.Virol. 65:4735,1991). Competition binding experiments, where the attachment of radioactively labelled CAV-9 to GMK cells was analyzed in the presence of an excess of other enteroviruses, revealed that an other RGD-containing enterovirus, echovirus 22, was sharing the receptor specificity with CAV-9. The role of RGD naturally pinpoints towards integrins as candidate receptor. For further characterization of the receptor protein(s) several antibodies to integrin components were screened for a possible protective effect towards CAV-9 infection. The purification of CAV-9 receptor was done by using affinity chromatography. The detergent extracts of ^{125}I-labelled GMK cells were chromatographied on Sepharose-linked to purified CAV-9. The column was eluted with synthetic peptide which includes the virus specific cell attachment sequence. Two polypeptides with estimated molecular weights 105 and 125 kDa were detected from eluted fractions under nonreducing conditions. The peak fraction was found to block CAV-9 infection. Further characterization of the protein(s) is in progress.

B 133 UTILIZATION OF CHOLESTEROL AND CHOLESTEROL ESTER FOR ADHERENCE BY *PSEUDOMONAS AERUGINOSA*, Katherine S. Rostand and Jeffrey D. Esko, Department of Biochemistry, University of Alabama at Birmingham, 35294

Pseudomonas aeruginosa is an opportunistic pathogen responsible for infections in many hospitalized patients and for chronic bacterial colonization of cystic fibrosis airways. Cell surface glycoconjugates and mucins in airway secretions are putative receptors mediating *Pseudomonas* adherence to the epithelium and binding to mucous secretions . We have utilized glycosylation mutants of Chinese hamster ovary cells to attempt to define native receptors for a clinical isolate of *Pseudomonas aeruginosa*. Adherence to wildtype CHO cells was maximal after 40 min and binding saturated at 10 bacteria/CHO cell. When mutants defective in proteoglycan synthesis, N-linked and O-linked glycosylation, and glycolipid synthesis were tested adherence to mutant cells was the same as to wildtype. Extraction of wildtype monolayers with Triton X-100 or ethanol greatly diminished adherence and binding of *Pseudomonas* to factors in the extracts could be demonstrated. These factors were stable to alkali, heat and proteolysis. An ethanol extract of wildtype CHO cells was fractionated on silica gel 60 and fractions containing the binding activity were pooled. TLC showed that the material behaved like cholesterol and cholesterol ester. NMR spectroscopy confirmed the identity of the active components. We suggest that plasma membrane cholesterol may mediate *Pseudomonas* adherence to airway epithelial cells. Both cholesterol and cholesterol esters are major components of airway secretions. Reduced clearance of such secretions in cystic fibrosis lungs could promote colonization by *Pseudomonas*.

B 134 FUNCTIONS OF GENES IN MORPHOGENESIS OF CS1 PILI, June R. Scott, Alexander Karakashian, Lawrence R. Melsen, Jeff Wakefield and Barbara Froehlich, Department of Microbiology and Immunology, *Emory University School of Medicine*, Atlanta, GA, 30322

The CS1 pili on the surface of many strains of enterotoxigenic Escherichia coli (ETEC) are thought to be important for colonization of the human intestine. We have cloned and sequenced a piece of DNA which causes E. coli K12 to produce CS1 pili. The major pilin protein is encoded by cooA, the second gene in the cluster (Perez-Casal, Swartley, and Scott. 1990. Infect. Immun. 58: 3442-3444). A search .of the genbank database indicates that the sequences of cooB (the first gene), cooC (the third gene) and cooD (the fourth gene) have no homology with any proteins other than those predicted from the DNA sequence of CFA/I, a serologically different type of pilus found on other human ETEC strains. The coo genes produce proteins of approximately the expected sizes when transcribed and translated in vitro. The CooB and CooC proteins are required for assembly of the pili. However, they do not influence the stability of the pilin protein and thus do not appear to be chaperonins. An insertion mutation in cooB is polar on cooA and an insertion in cooC is polar on CooD. Preliminary evidence suggests that cooD is also needed for assembly but not for stability of the CooA pilin protein.

B 135 YADA MEDIATES SPECIFIC BINDING OF ENTEROPATHOGENIC *YERSINIA ENTEROCOLITICA* TO HUMAN INTESTINAL SUBMUCOSA. Mikael Skurnik, Yasmin El Tahir, Marja Saarinen, Sirpa Jalkanen, and Paavo Toivanen. Turku Centre for Biotechnology, and Department of Medical Microbiology, Turku University, SF-20520 Turku, Finland

The binding of live *Yersinia enterocolitica* to frozen sections of human intestine was investigated qualitatively by monitoring the binding of bacteria using Gram- or immunoperoxidase staining, as well as quantitatively by a new EIA-on-slide method. When YadA-expressing and non-expressing variants of different *Y. enterocolitica* serotypes, *yadA* mutants, and *Escherichia coli* clones were tested in the binding assays, it became evident that the binding to frozen sections was YadA-mediated. The YadA-mediated binding of bacteria occurred mainly at the submucosal layer of the intestinal wall, and only to a limited extent at the mucosal layer; there mostly to the mucin threads. In addition, partially purified YadA bound to frozen sections with a pattern similar to that of intact bacteria. Collagen, laminin, or partially purified YadA alone did not efficiently inhibit the YadA-mediated binding of bacteria; this was probably due to the multifunctional nature of YadA. However, a combination of collagen and laminin entirely inhibited the binding. These results imply that YadA may be involved in the interactions with the extracellular matrix molecules subsequent the invasion of the intestinal tissue.

B 136 FUNCTIONAL ANALYSIS OF A GLYCO-CONJUGATE BINDING PROTEIN EXPRESSED ON THE SURFACE OF INFECTIVE *LEISHMANIA*

Deborah F. Smith, Helen M. Flinn, Suzanne Gokool and Desikan Rangarajan, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK.

Protozoa of the genus *Leishmania* are causative agents of the broad spectrum of human diseases termed the leishmaniases. During the life cycle of these parasites, extracellular, flagellated, infective organisms are transmitted between mammalian hosts by blood-feeding female sandflies. Within the host, the parasites enter and survive intracellularly, as aflagellated forms within macrophages.

Characterisation of specific molecules associated with parasite virulence has been aided by the use of 'reverse genetics' to identify genes that are expressed either uniquely or at increased levels in infective stages. Using this approach, several novel sequences have been identified. Recombinant fusion proteins expressed from the open reading frames of these genes have allowed the generation of specific antibodies for analysis of protein structure and function. One of the proteins, a proline-rich, hydrophilic molecule, is located on the surface of infective, extracellular parasites where it interacts with the major surface glyco-conjugate coat, lipophosphoglycan. The role of this protein in parasite infectivity is under investigation, using both biochemical and genetic techniques.

B 137 TRANSLOCATION ACCROSS THE MEMBRANE OF EUKARYOTIC CELLS OF A HYBRID PROTEIN BETWEEN YopE OF YERSINIA ENTEROCOLITICA AND THE FIRST 400 AMINO ACIDS OF ADENYLATE CYCLASE OF BORDETELLA PERTUSSIS, Marie-Paule Sory and Guy R. Cornelis, Microbial Pathogenesis Unit, University of Louvain, Medical School, 1200 Brussels, Belgium.

The escape of Y. enterocolitica from the primary immune response of the host depends on the Yop proteins. YopE, a cytotoxin of 25 kDa, provokes complete destruction of the actin microfilaments. YopH is a phosphotyrosine phosphatase that inhibits phagocytosis by macrophages. Interactions of Yops with the eukaryotic cells suggest that they are, at some stage, translocated from outside the cells to the cytoplasm or from one compartment to another. To address this question, we constructed a fusion protein between the first 130 amino acids of YopE and the first 399 amino acids of the adenylate cyclase of Bordetella pertussis. This region of adenylate cyclase only contains the catalytic region and the calmodulin binding site. The chimeric protein was secreted by recombinant bacteria and still catalyzed the hydrolysis of ATP to cAMP. The pathogenicity of the recombinant strain was strongly decreased as compared to the parental strain. This resulted from its growth thermosensitivity phenotype. Infection of HeLa cells or macrophages with the recombinant strain induced an increase of cAMP inside both cell lines. This response still occured in cytochalasin D-treated cells, in spite of the absence of internalized bacteria. However, only Inv+ and live bacteria induced it. Moreover, the cAMP increase was not observed when using bacteria deficient in the secretion of Yops. We concluded that extracellular bacteria can inject Yop proteins in the cytoplasm of target cells.

B 138 HERPESVIRUS SAIMIRI ENCODES FUNCTIONAL INHIBITORS OF BOTH THE C3 CONVERTASE AND MEMBRANE ATTACK COMPLEX STEPS OF THE COMPLEMENT CASCADE, Stephen P. Squinto, William L. Fodor, Scott A. Rollins, Jens-C. Albrecht#, Bernard Fleckenstein#, and Russell Rother, Alexion Pharmaceuticals, Inc., New Haven, CT 06511 and #Friedrich-Alexander Universitat, Erlangen, Germany

Herpesvirus saimiri is a lymphotrophic herpesvirus that induces lymphocyte transformation in vitro and causes lymphomas and leukemias in new world primates. Nucleotide sequence analysis of the HVS genome revealed two open reading frames with significant homology to known human complement regulatory molecules. The HVS-15 gene encodes a predicted protein with sequence and overall structural homology to the human terminal complement inhibitor protein, CD59. The HVS-CCPH gene exhibits sequence and structural homology to a family of complement regulatory proteins that all function to inhibit human complement at the C3 convertase step in the complement cascade. Proteins in this gene cluster (RCA; regulators of complement activation) include CD21 (CR2), CD35 (CR1), CD46 (MCP), CD55 (DAF), C4bp, and Factor H. Additionally, the major secreted protein of vaccinia virus, gp35, shares structural motifs with the RCA family of proteins and its ability to block C3 convertase activity has been associated with its role in productive viral infectivity. To determine the potential complement regulatory activity of the HVS-15 and HVS-CCPH proteins, cDNAs encoding these molecules were stably transfected into mouse Balb/3T3 cells. Complement inhibitory activity was assessed using a dye release assay on control and transfected cells challenged with antibody and human complement. Cells expressing HVS-15 were significantly protected from lysis by both human and rat complement. The complement regulatory activity of HVS-15 occurred after C3b deposition indicating terminal complement inhibition and like CD59, HVS-15 activity was PI-PLC sensitive indicating the HVS-15 is anchored by a glycophospholipid moeity. HVS-CCPH was also able to inhibit human and rat whole serum complement-mediated lysis and the target of the complement cascade for HVS-CCPH was C3 convertase. These data are the first to demonstrate that a pathogenic mammalian virus can harbor two functional complement inhibitors.

B 139 COMPLEMENTATION OF A *SALMONELLA ENTERITIDIS tolC* MUTANT DEFECTIVE FOR TISSUE CULTURE INVASION AND ATTENUATED FOR VIRULENCE. BARBARA J. STONE and VIRGINIA L. MILLER, Department of Microbiology and Molecular Genetics, UCLA, Los Angeles, CA 90024.

Salmonella enteritidis, a causative agent of gastroenteritis, invades and transverses the intestinal epithelium to reach the primary site of multiplication, the reticuloendothelial system. A mutation generated by Tn*phoA* insertion into the *S. enteritidis* chromosome led to a defect in invasion of CHO, MDCK, and HEp-2 tissue culture cells. The mutant, SM6, is severely attenuated for virulence in a mouse model and has been found to be defective in colonization of the Peyer's patches. A *S. enteritidis* DNA library was constructed, pooled, and mated into SM6. Clones which complemented the invasion defect were enriched for by passage of SM6 carrying the cosmid library through tissue culture cells. This led to the isolation of the wild type locus corresponding to the region of the transposon insertion in SM6. From subclone analysis of the complementing cosmid, a 3.5kb region of chromosomal DNA was identified which was necessary but not sufficient for complete complementation of the wild type phenotype in tissue culture. Sequence analysis of the 3.5kb region revealed that the transposon insertion of SM6 is in the *Salmonella enteritidis* homologue of *tolC*. TolC is necessary for the secretion of α-hemolysin in *E. coli*. A 7.5kb region of DNA containing the *tolC* homologue was identified which conferred to SM6 higher than wild type invasion levels in tissue culture cells. The locus cloned may therefore contain more than one gene which is required by *Salmonella enteritidis* for effective epithelial cell entry.

B 140 PHASE VARIATION OF HAEMOPHILUS INFLUENZAE FIMBRIAE, Loek van Alphen, S. Marieke van Ham, Frits R. Mooi, Jos van Putten, Depm of Medical Microbiology, University of Amsterdam, The Netherlands and Max Planck Institut für Biologie, Tübingen, Germany.

The expression of H.influenzae fimbriae is subject to revesible phase variation between three expression levels. This phenomenon is controlled at the transcriptional level of two divergently oriented genes, hifA and hifB, encoding the major fimbrial subunit and the fimbrial chaperone, respectively. The hifA and hifB promotor regions were found to be clustered through an almost complete overlap with a variable DNA backbone of TA units. Variation in the number of units changes the normally strictly constraint spacing between the -35 and -10 sequences and controls the bidirectional transcription initiation, thus forming a novel mechanism directing multiple gene transcription.

B 141 A MENINGITIS-SPECIFIC ANTIGEN, DEFINED BY A RUBELLA MONOCLONAL ANTIBODY, CONTAINS hMCP-1& 3 SEQUENCES

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Rubella Virus (RV) is known to cause a mild meningomyelitis. Whole virus was employed to construct monoclonal antibodies (Mab's) to RV. One of these, designated RV1, was characterized using Western Blot analyses of whole virus. RV1 was found to bind to the 45 Kd glycosylated, membrane-associated E2 protein and to the 30 Kd core protein. The Macintosh DNA and Protein ("Macintosh DNASIS Pro") Sequence Analysis System was employed to define Homologous Antigenic Sequences (HAS's) in both the envelope and core proteins. Each homologous sequence is composed of seven amino acids.

The RV1 Mab was found to cross-react with proteins in several other meningitis-causing agents including: **Hemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae,** and **Listeria *monocytogenes*** (which together account for more than 85% of all bacterial meningitis in the United States), and HIV1. A family of HAS's was found to be common to all of the above-mentioned infectious agents.

All etiological agents of meningitis studied here express HAS's on their surfaces, and infect monocytes/macrophages, prior to entering the central nervous system (CNS). These highly conserved, meningitis-specific HAS's have also been identified in 2 variants of the human Monocyte Chemoattractant Factor (hMCP-1 and hMCP-3). This factor is thought to recruit lipid-scavenging monocytes implicated in "foamy cell"and plaque formation near fatty streaks in arteries. These same lipid-scavenging monocytes may also routinely enter the lipid-rich CNS to perform maintenance functions related to normal turn-over and repair. Therefore, the meningitis-related function of HAS's may be to recruit these specific monocytes for subsequent infection, thereby ensuring the entry of the infectious agents into the CNS.

B 142 HLA B27 MODULATES INTRACELLULAR KILLING OF SALMONELLA IN TRANSFECTED L CELLS, Mika Virtala, Juha Kirveskari and Kaisa Granfors, National Public Health Institute, Department in Turku, SF-20520 Turku, Finland

Several lines of evidence support the concept that bacteria or bacterial structures persist for a long time after acute infection in the patients developing reactive arthritis after Salmonella infection. First, persisting antibody responses against Salmonella LPS in sera and synovial fluids of patients with reactive arthritis suggested the persistence of corresponding antigens, too. Then, LPS structures were detected in inflamed joints and in peripheral blood, within mononuclear and polymorphonuclear phagocytes. It seems likely that the interaction between host and microbe is abnormal and inefficient in HLA B27 positive subjects in whom reactive arthritis develops.

In order to evaluate the role of HLA B27 in Salmonella-host interaction we examined intracellular killing of Salmonella in mouse L-cells (fibroblasts) transfected either with human β_2-microglobulin and HLA B27 or with human β_2-mg only (control cells). The B27- transfected and the control cells were incubated with Salmonella enteritidis for 2 h to allow the invasion, washed, and then incubated in the medium containing gentamycin to kill extracellular bacteria. The cells were lysed at 1 h, 1, 3, 6, 10 and 15 d to calculate intracellularly living bacteria.

HLA B27 expression does not modulate invasion of Salmonella into L cells; the numbers of living bacteria were always the same in the B27-transfected and in the control cells at 1 hour's incubation time. Thereafter, at the all different time points, the B27-transfected cells always contained more living Salmonellae than the control cells. The difference was increasing during the incubation time; at 6 days the B27-transfected cells contained 5-15 times more living bacteria than the control cells. At 10 days the corresponding ratio in preliminary experiments was close to 100.

These studies demonstrate that HLA B27 decreases intracellular killing of Salmonella in transfected L cells. This finding may have relevance to the persistence of bacteria in the HLA B27 positive patients developing reactive arthritis, and thus to the pathogenesis of reactive arthritis.

B 143 THE ROLES OF INVASIN, Ail AND YadA IN INVASION AND SURVIVAL OF *YERSINIA ENTEROCOLITICA IN VIVO*, Jeffrey C. Pepe, Marian R. Wachtel and Virginia L. Miller, Department of Microbiology and Molecular Genetics, UCLA, Los Angeles, CA 90024.

Several bacterial pathogens encode more than one invasion factor that may be required during specific stages of an infection. At least three unique invasion factors (Invasin, Ail and YadA) have been identified in *Yersinia enterocolitica*. It was recently shown that Invasin is vital for *Y. enterocolitica* to efficiently penetrate the intestinal barrier; however, *inv* mutants can cross the intestinal epithelium since they were recovered from deeper tissues. Presumably, penetration was occurring via alternate, less efficient invasion pathways encoded by *ail* and/or *yadA*. To investigate this question we examined *Y. enterocolitica* mutants defective for the following genes: *inv, ail, yadA, inv ail, inv yadA, ail yadA* and *inv ail yadA*. None of the four *inv* mutants were able to penetrate cultured epithelial cells as compared to wild-type; however, the *ail, yadA* and *ail yadA* mutants showed wild-type levels of invasion. In contrast to the *inv, ail, inv ail* and wild-type strains, which had similar LD_{50}'s in both orally (~10^6) and intraperitoneally (~10^3) infected mice, the four *yadA* mutants were severely decreased for virulence with both LD_{50}'s increased by $\geq 10^4$ CFU. All four *inv* mutants were unable to penetrate the intestinal epithelium *in vivo* 6 h after oral infection of BALB/c mice, consistent with the *in vitro* observations; thus, the *inv* mutation was dominant during the initial invasion step. In contrast, the *ail, yadA* and *ail yadA* mutants were as invasive as wild-type up to 18 h post-infection. Interestingly, later time points (32-40 h post-infection) revealed that the four *yadA* mutants were cleared from the Peyer's patches (PP). Furthermore, the *ail* mutant was unaffected for survival after 40 h, but the *ail yadA* mutant was decreased even more in survival than the single *yadA* mutant. These data indicate that: 1) *inv* is necessary for the initial entry into PP; 2) that *yadA* is required for survival in PP; and 3) that *ail* contributes to survival in PP.

B 144 IDENTIFICATION AND CLONING OF A NOVEL PROTECTIVE ANTIGEN FROM *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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Corynebacterium pseudotuberculosis is an intracellular bacterial pathogen that causes caseous lymphadenitis (CLA), a chronic disease of sheep and goats, characterised by necrotic lesions in peripheral lymph nodes and the lung. A phospholipase D exotoxin and a cell-wall associated lipid are established virulence factors. Vaccines based on crude and purified toxoid have given significant but variable levels of protection, with evidence that additional antigens are required for solid immunity to CLA.

Analysis of the local immune response to a challenge infection identified a novel 40kD antigen from *C. pseudotuberculosis* . Vaccination of sheep with purified 40kD antigen incorporated into aluminium hydroxide adjuvant gave high levels of protection, equivalent to that obtained with a commercially available complex vaccine

Cloning of the 40kD gene was achieved using oligonucleotides derived from amino acid sequence data from the N-terminus and CNBr-peptides to generate a PCR probe, which was used to screen a *C.pseudotuberculosis* genomic library. Sequence analysis of the ORF revealed a 31 amino acid secretory leader peptide, with a typical Gram +ve cleavage motif. The ORF of the 40kD gene minus the leader sequence was expressed to high levels in *E.coli* .

Cloning and expression of a major protective antigen of *C. pseudotuberculosis* will assist in the development of an efficacious recombinant vaccine against CLA.

B 145 THE EFFECT OF MODIFIED LACTOFERRIN PROTEINS ON THE GROWTH OF ENTEROPATHOGENIC STRAINS OF BACTERIA. Pauline P. Ward, and Orla M. Conneely. Dept. Cell Biology, Baylor College of Medicine, Houston, Tx, 77030.

Lactoferrin is an iron-binding glycoprotein that is present in external secretions such as milk, tears, saliva, intestinal mucus and intracellularly in the secondary granules of polymorphonuclear leucocytes. Several physiological functions have been ascribed to lactoferrin including regulation of iron absorbtion in the small intestine, stimulation of systemic immune response and protection against microbial infection. The antibacterial functions of lactoferrin are contributed by two seperate domains of the protein. The first is a bacteriostatic function provided by two repeated iron-binding domains which bind iron with very high affinity thereby depriving bacteria of this essential growth nutrient. The second antimicrobial domain has a direct bactericidal function and is located in the N-terminus in a region distinct from the iron-binding domains. This bactericidal domain causes the release of lipopolysaccharide from the outer membrane of a broad spectrum of bacteria.

While lactoferrin exhibts a potent antimicrobial effect at potential sites of infection in the body, it contributes to the virulencence of certain pathogenic strains of bacteria by its ability to recognise bacterial outer membrane receptor proteins that minic the intestinal receptor for lactoferrin in that they can bind and utilize iron-loaded lactoferrin.

We have generated deletion and single amino-acids point mutants of the hLF cDNA to identify the region of lactoferrin which interacts with the bacterial receptor and. to generate lactoferrin mutants with altered antimicrobial activities. The mutant lactoferrins were expressed and purified from Aspergillus oryzae using the previously described expression system for the production of biologically active recombinant lactoferrin in A. oryzae. The antimicrobial properties of these mutants with respect to the growth of two enteropathogenic strains of bacteria will be discussed.

B 146 THE EFFECT OF COLONY OPACITY VARIATION ON PNEUMOCOCCAL COLONIZATION AND ADHESION

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When viewed with oblique, transmitted light on a transparent surface, encapsulated isolates of*Streptococcus pneumoniae* form sectored colonies and are heterogeneous in appearance because of variation in opacity. There is spontaneous phase variation *in vitro* between at least four distinct phenotypes at frequenies of 10^{-3} to 10^{-6}/generation. The ability to detect differences in opacity varies according to serotype, but variation is independent of expression of capsule. Electron-microscopy shows that the capsule and cell wall are more loosely associated with the cell in the more opaque forms. The biochemical basis of variation in opacity is not yet resolved. Surface-labelling with biotin, however, demonstrates proteins in the opaque variant which are not labelled in the transparent variant.

An infant rat model has been used to compare the ability of different variants of serotypes 9V and 18C, which vary at higher and lower frequencies respectively, to colonize the nasopharynx. Colonization by the transparent variant of either serotype is efficient and stable. There was no significant colonization by the opaque 18C variant. In the case of the 9V opaque variant, the nasopharynx is colonized after *in vivo* phenotypic switching to the transparent form. Opaque and transparent forms are able to interact with cultured type II pneumocytes and HUVECs. However, the transparent form is Δ25% better at adhesion than the opaque variant for both target cells. Organisms displaying the transparent phenotype appear to be better suited for adhesion and colonization. A biological role for the opaque phenotypes could not be detected by these studies. Results suggest that phase variation which is apparent as differences in colony morphology may provide insight into the interaction of the pneumococcus with its host.

B 147 ROLE OF CRYPTOSPORIDIUM PARVUM MICROTUBULES IN HOST CELL INVASION, Peter M. Wiest, Krista Dong, Joan H. Johnson, Kim Boekelheide, and Timothy P. Flanigan, Departments of Medicine and Pathology, Brown University, Providence, RI 02906.

Cryptosporidium parvum is an protozoan parasite that causes diarrhea in patients with AIDS. No therapy is effective in the treatment of cryptosporidiosis. The role of C. parvum microtubules in host cell invasion was determined by infecting HT 29.74 cells with oocysts in the presence of microtubule inhibitors and examining the rate of infection 24 hr later. The number of intracellular parasites decreased by 77% ($P<0.01$) in cells treated with colchicine (10^{-4}M) as compared to controls. A significant reduction in infectivity was also found when oocysts were pretreated with colchicine for 15 min and used to infect host cells in drug-free medium. Pretreatment of host cells, however, did not inhibit infectivity. Vinblastine also decreased significantly the rate of infection. Anti-microtubule drugs did not block sporozoite excystation. Indirect immunofluorescence demonstrated that the anti-β-tubulin monoclonal antibody stained permeabilized sporozoites but not organisms treated with colchicine. This antibody recognized C. parvum proteins (54, 58 kDa) by immunoblot analysis. [3H]-colchicine bound C. parvum extracts (3,136 ± 936 dpm) as compared to controls (599 ± 156 dpm) suggesting tubulin binding. These data suggest that C. parvum microtubules are critical in host cell invasion and may represent pharmacological targets for treatment of cryptosporidiosis.

B 148 CLONING OF O-ANTIGEN BIOSYNTHESIS GENES OF *Y. ENTEROCOLITICA* SEROTYPE O:8.

Lijuan Zhang and Mikael Skurnik. Turku Centre for Biotechnology, and Department of Medical Microbiology, Turku University, SF-20520 Turku, Finland

O-antigen is one of the virulence factors of *Y. enterocolitica*. A chromosomal cosmid library of *Y. enterocolitica* serotype O:8 (YeO8) was constructed in *E. coli* DH1 to characterise the biological role of O-antigen in the pathogenesis of *Yersinia* infections and in its post-infectious complications. A YeO8 O-antigen specific monoclonal antibody, FU26-IFI-1 was used for the screening of the library by colony immunoblotting and two positive clones were picked up. Preliminary deletion analysis showed that *E. coli* harbouring the recombinant plasmid, pLZ6010, which carries an insert of 20 kb of YeO8 chromosomal DNA, expressed YeO8 specific O-antigen on the cell surface. This was shown by immunoblotting and LPS isolation. Further characterization of this gene cluster is being carried out.

Molecular Interactions Between Viruses and Host Cell Membranes; Exploitation of Host Cell Organelles in Early Infection

B 200 HOW VIRAL ENVELOPE GLYCOPROTEINS NEGOTIATE THE ENTRY OF GENETIC MATERIAL INTO THE CELL

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Specific fusion of biological membranes is a central requirement for many cellular processes. Biological fusion events are strongly regulated and coordinated presumably by specific membrane fusion-inducing proteins. The best studied examples are viral envelope proteins. The fusion process involves a range of steps before the final merging of membranes occurs. Our studies deal with a number of key questions concerning the fusion process such as: How does triggering the event by a pH or temperature change, or receptor binding affect conformation of the env glycoprotein?; How do they mediate adhesion at the site at which fusion is to occur and movement of membranes into apposition?; Can we identify intermediate fusion steps or structures?; What sorts of molecular rearrangements occur after before, during and after the fusion event? Those questions are approached by developing kinetic assays for fusion of fluorescently-labeled virus with a variety of target membranes using spectrofluorometric and video microscopic techniques. We find that the fusion is a rather complex process in which the system has to undergo series of multiple steps before the final fusion event occurs. Those steps are characterized as molecular processes such as conformational changes, aggregation, lipid-protein interactions, fusion pore formation and pore widening.

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B 201 ISOLATION OF A CELL MUTANT RESISTANT TO VACCINIA VIRUS KILLING USING A RETROVIRAL PROMOTER TRAP VECTOR

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To improve the efficiency of insertional mutagenesis we have developed retroviral vectors that confer selectable phenotypes when viruses integrate into expressed genes. These vectors contain coding region of selectable markers (i.e. histidinol dehydrogenase (His), hygromycin transferase (Hygro), β-galactosidase (β-gal) and HSV thymidine kinase (TK)) inserted into the U3 region of viral 3' long terminal repeat (LTR). Thus, when viruses replicate, duplication of the LTR places marker sequences in the 5' LTR just 30 nucleotides next to the flanking cellular DNA. Selection for marker gene expression generates cell clones in which proviruses integrated downstream of transcriptionally active promoters, disrupting cellular genes in the process.

In addition , These promoter trap vectors have been shown to be effective mutagens and can inactivate cellular genes at a frequency of 1×10^{-5}.

We have used U3His virus as an insertional mutagen to identify cell mutants resistant to vaccinia virus(VV) infection. Chinese hamster ovary (CHO) cells were infected by U3His and 10^5 His^r clones were isolated, and subsequently infected by vaccinia virus. One clone resistant for vaccinia virus killing was isolated. It appears that in this mutant VV5-4 replication of VV genome is blocked. Cellular promoter trapped by U3His in VV5-4 was isolated and a 5Kb transcript was detected in Northern blot analysis. Experiments are in progress (1). to isolate the cDNA interrupted by provirus integration (2). to express cDNA in mutant cells to complement the phenotype.

B 202 INTERACTION OF TOXOPLASMA PARASITOPHOROUS VACUOLE WITH THE HOST CELL NUCLEUS IN VERO CELLS, Halonen,S.K. and E. Weidner, Dept. of Zoology, Louisiana State University, Baton Rouge, LA 70803

Toxoplasma actively invade their host cells, residing within a membrane compartment that moves through the cytoplasm and is typically found near the host cell nucleus within 30-60 minutes after invasion. The vacuole usually retains this juxtanuclear position throughout intracellular infection and the host cell nucleus often appears "indented" due to the close opposition of the enlargening vacuole. Furthermore, the vacuole retains this juxtanuclear position during mitosis. We have studied the interaction of the parasitophorous vacuole with the host cell intermediate filaments in Vero cells. We have found the vimentin type intermediate filaments (IF) begin to associate with parasitophorous vacuole within the first hour after invasion, occuring coincidentally with the arrival of the vacuole at the host cell nucleus. As the parasite grows, the IF's emananting from the host cell nuclear surface envelope and encage the enlargening parasitophorous vacuole, appearing to restrain the parasitophorous vacuole to the nuclear surface. Furthermore disassembly of the IF's causes displacement of the parasitophorous vacuole from the host cell nuclear surface suggesting the vimentin type IF's serve to "dock" the vacuole to the host nucleus. The retension of the vacuole to the nucleus during mitosis, may be due to interaction of the vacuole with the lamin type intermediate filaments associated with the nuclear membrane.

B 203 CHARACTERISATION OF THE SITE OF ANTIGEN PROCESSING IN *LEISHMANIA* INFECTED MACROPHAGES, Robert A. Harris, Dennis M. Dwyer* and Paul M. Kaye, Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, WC1E 7HT U.K. and *Laboratory of Parasitic Diseases, N.I.A.I.D., National Institutes of Health, Bethesda, MD 20205

The protozoan parasite *Leishmania* is taken up by its host macrophage by phagocytosis. Previous functional and immuno-EM studies suggested that the parasitophorous vacuole (PV) formed after entry was the site of initial proteolysis of dead parasites for antigen presentation, but that class II binding of processed antigen occurred in another (endosomal?) compartment (Lang & Kaye, *Eur J Immunol.* 1991 **21**:2407). Recent studies by others, examining the phagocytosis of latex beads have suggested, however, that phagosomes and endosomes both meet in a common tubulo-reticular structure (Rabinowitz *et.al. J.Cell Biol.* 1992 **116**:95). We have, therefore, analysed in more detail the nature of the *Leishmania donovani* PV.

Similar to other *Leishmania sp.*, the PV formed around *L.donovani* is stained for both macrosialin and lamp1, these markers being acquired within 20 min of ingestion. However, in contrast to other species, the *L.donovani* PV is inaccessible to fluid phase tracers, and co-challenge with heterologous particles, such as zymosan, does not result in shared occupancy of PVs. Newly synthesised class II molecules may, however, be directed into the PV after export from the ER/Golgi. Furthermore, the *L.donovani* secretory acid phosphatase (SAcP) is progressively released from the PV over time. The apparent selectivity of intracellular trafficking in and out of the *L.donovani* PV and the ubiquitous distribution of SAcP may have important implications for the presentation of parasite antigens by the infected host cell.

B 204 INFLUENZA VIRUS EFFECTS ON MACROPHAGE ENDOCYTOSIS ASSAYED USING VIDEO MICROSCOPY
R. Joel Lowy, Physiology Department, AFRRI, Bethesda, MD 20889

An important part of infectivity and virulence for many pathogens is the circumvention or disruption of normally functioning intracellular vesicle trafficking pathways e.g. inhibition of phagolysosomal fusion or cytoplasmic entry prior to delivery to lysosomes. Influenza virus (IV) in addition to its own pathogenic effects can increase secondary infections probably in part by inhibition of phagocyte function. Digitally enhanced fluorescence video microscopy (DFVM) has been successfully used to examine and quantitate early events associated with IV cell entry including single viral particle fusion (Lowy et al. *PNAS* 87:1850, 1991) and movement of envelope molecules subsiquent to fusion (Lowy et al., *Biophys. J.* 64c:A188, 1993). Our current experimentation is directed towards using quantitative microscopy to understand the early effects of IV entry on vesicle trafficking in J774.1 cells, a well characterized murine macrophage cell line. DFVM measurements using Texas Red Dextran (TR-Dex) showed fluid phase uptake kinetics, using small numbers of cells, were similar to those performed with macroscopic fluorimetric methods. Attached J774 cells were pre-activated or sham exposed to 5 ug/ml LPS for 18 hours, then pre-or sham exposed to IV for 30 min, and washed to remove surface bound virus. Fluid phase uptake was assayed using a 60 min uptake of TR-Dex. IV exposure decreased TR-Dex uptake in both LPS treated cells and non-treated cells; the average percent of control being 42 ± 12 and 66 ± 3, respectively. The results are similar to those previously reported for PMNLs post IV exposure and suggest that IV alters endocytic processes in phagocytes by an unknown mechanism(s). The results also demonstrate the feasibility of using DFVM to examine the endocytic activity of macrophages during early envelope virus entry.

B 205 RHO GTP-BINDING PROTEINS ARE THE TARGETS OF CYTOTOXIC NECROTIZING FACTOR TYPE 2 PRODUCED BY VIRULENT *ESCHERICHIA COLI*. Eric Oswald, Motoyuki Sugai, Agnès Labigne, Henry C. Wu, Patrice Boquet and Alison D. O'Brien. Uniformed Services University of the Health Science, MD 20814, USA, Hiroshima University School of Dentistry, Hiroshima 734, Japan, Institut Pasteur, 75015 Paris, France.

Cytotoxic necrotizing factor type 2 (CNF2) produced by *Escherichia coli* strains isolated from intestinal or extraintestinal infections is a dermonecrotic toxin of 110 kDa. In this study, the gene encoding CNF2 was cloned from a large plasmid carried by a strain isolated from a lamb with septicemia. Hydropathy analysis of the deduced amino acid sequence revealed a largely hydrophilic protein with two potential hydrophobic transmembrane domains. The N-terminal half of CNF2 showed significant homology (27% identity and 80% conserved residues) to the N-terminal portion of dermonecrotic toxin of *Pasteurella multocida*, a potent mitogen which is thought to facilitate the coupling of heterotrimeric GTP-binding protein to phospholipase C. Methylamine protection experiments and immunofluorescence studies suggested that CNF2 enters the cytosol of the target cell through an acidic compartment and induces the reorganization of actin into stress fibers. Since the formation of stress fibers in eucaryotic cells involve Rho, we radiolabeled these small GTP-binding proteins from CNF2-treated and control cells with a Rho-specific ADP-ribosyl transferase. The [$AD^{32}P$]-ribosylated Rho proteins from CNF2-treated cells migrated slightly more slowly in SDS-PAGE than did the labelled proteins from the control cells. This shift in mobility of Rho proteins in SDS-PAGE was also observed when CNF2 and the RhoA protein were coexpressed in *E. coli*, a finding which clearly demonstrates that the modification of Rho proteins by CNF2 does not require prior C-terminal modification and processing by enzymes unique to eucaryotic cells. Because PMT and CNF2 share several common characteristics, we propose that the CNF toxins and PMT are members of a heretofore unrecognized family of toxins which modify GTP-binding proteins.

B 206 EXPOSURE OF ENTEROVIRUSES TO INTESTINAL TRYPSIN, Liisa Piirainen, Merja Roivainen, Antero Airaksinen and Tapani Hovi, National Public Health Institute, Helsinki, Finland

During replication in the gut all enteroviruses are exposed to trypsin and other host proteolytic enzymes in intestinal fluid. Previously we have shown that exposure of coxsackievirus A9 to intestinal trypsin resulted in selective cleavage of a small fragment from the C-terminal end of VP1. After cleavage the virus was infectious but its receptor specificity was altered. Most strains of type 2 and 3 polioviruses are also known to be sensitive to intestinal trypsin. In these cases, however, the site of cleavage is a single surface exposed loop of VP1 capsid protein that is a central part of the designated antigenic site 1. This cleavage results in an altered antigenicity of virus as well as in an enhanced rate of uncoating of the cell-bound virus. Now several other enteroviruses were tested for their susceptibility to intestinal trypsin. All trypsin-treated viruses were infectious. A possible alteration in early virus-cell interactions were further studied by analyzing one step growth curves, the rate of uncoating and in some cases the rate of RNA synthesis in cell cultures infected with intact or trypsin-treated viruses.

B 207 Chlamydia trachomatis does not require an acidic pH for its development and utilizes microtubules for endosome fusion. N. Schramm and P.B. Wyrick. Dept. Microbiology, Univ. North Carolia School of Medicine, Chapel Hill, NC 27599

Chlamydia trachomatis is an obligate intracellular bacterium which invades eucaryotic cells and remains within a membrane bound vesicle during its complex developmental cycle. Following endocytosis, chlamydia-containing endosomes fuse together in the host cell cytoplasm to form one large inclusion which expands as the bacteria divide. The endosome evades fusion with host cell lysosomes (Eissenberg & Wyrick, 1989, Infect. Immun.32, 889-896), however, the pH of this vesicle has yet to be determined. Inhibition of the Na^+/K^+ATPase in host cells with ouabain, which leads to a rapid pH drop in early endosomes (Cain et al,1989,PNAS 86,544-548), inhibits chlamydial inclusion formation, whereas, treatment of chlamydiae-infected HEC-1B cells with the H^+ ATPase inhibitor, N-ethylmaleimide, has no effect on inclusion formation. These results show that chlamydiae do not require acidification of the endosome for development. Current efforts to directly measure the pH of the inclusion using the pH sensitive probe SNAFL are underway. The mechanism utilized by chlamydiae for vesicular fusion to form one mature inclusion is currently unknown. The microtubule disrupting drugs colchicine and nocodazole were used in order to determine whether inclusion fusion requires polymerized microtubules. Chlamydiae- infected HEC-1B cells treated with these drugs showed a decrease in inclusion fusion.

Strategies Used by Microorganisms to Cross Epithelial Barriers; Inflammation: Cellular and Extracellular Inducers-Methods of Intervention

B 300 MAST CELL DEGRANULATION AND BACTERIAL PHAGOCYTOSIS TRIGGERED BY TYPE 1 FIMBRIATED *ESCHERICHIA COLI*, Soman N. Abraham, Elaine Ross, Teruo Ikeda and Ravi Malaviya, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

Although adhesive fimbriae are known for their role in initiating bacterial infections on various mucosal surfaces, their role in inflammation is less well recognized. The interaction of type 1 fimbriae of *E. coli* with mouse mast cells was investigated. Mast cells are inflammatory cells which are likely to play a pivotal role in host defense against bacterial infections because they are (i) selectively found in the lining of mucosal surfaces, skin and around blood vessels and (ii) replete with potent inflammatory mediators. Type 1 fimbriated, but not nonfimbriated, *E.coli* induced mast cell degranulation and mediator release including histamine. The magnitude of mast cell degranulation and histamine release was directly proportional to the number of adherent type 1 fimbriated bacteria on the cell surface. The interaction with type 1 fimbriae also triggered mast cell phagocytosis and killing of some of the adherent bacteria. Mast cell bactericidal activity appeared to involve release of superoxide anions and acidification of phagocytic vacuoles. Inert beads coated with purified FimH, the mannose binding moiety located at the tips of type 1 fimbriae, activated mast cells to the same level as type 1 fimbriated *E.coli*. Further, FimH-coated but not bovine serum albumin-coated beads were internalized by the mast cells. These findings indicate that the determinant on type 1 fimbriae responsible for activating mast cells was FimH and, furthermore, this moiety is likely to play a key role in modulating bacterial inflammation in the host.

B 301 SELECTION OF S. TYPHIMURIUM GENES EXPRESSED SPECIFICALLY WITHIN INTESTINAL EPITHELIAL CELLS. Craig Altier and Russell Maurer, Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, OH.

S. typhimurium factors required for survival within and passage through intestinal epithelium are poorly characterized. We have sought candidates for such factors using a genetic screen for promoters that are expressed at a lower rate in Salmonella grown on laboratory media than in Salmonella resident within Caco-2 epithelial cells in culture. The screen utilizes the site-specific recombination system (lox-cre) from phage P1. Two lox sites are placed in the Salmonella chromosome bracketing markers for kanamycin resistance and sucrose sensitivity. A promoterless cre gene on a plasmid is fused to random small fragments of Salmonella DNA. When transcription of cre takes place, the lox sites recombine, leading to a kanamycin-sensitive, sucrose-resistant phenotype. Bacteria carrying cre plasmids in which expression is constitutive are killed by selection on kanamycin. The surviving cells are allowed to invade a Caco-2 monolayer, and sucrose-resistant individuals are recovered from the intracellular population. To date, we have recovered and begun to analyze nine distinct chromosomal fragments. Typically, for any one of these fragments, expression of sufficient cre to elicit recombination occurs in approximately 1% of cells grown in media, and in 90-99% of cells recovered from the intracellular population. Among the fragments so far isolated are a region of the oligopeptide permease operon (opp) and an ORF known to be associated with spermidine production in E. coli. Several novel loci have also been identified. This approach may be useful for investigation of other stages of pathogenesis of Salmonella and potentially of other microorganisms.

B 302 TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN-6 IN INFECTION OF HUMAN MACROPHAGES BY *BRUCELLA* SPP., Emmanuelle Caron, Stephan Köhler, Thierry Peyrard, Jacques Dornand, Jean-Pierre Liautard. INSERM U-65, Université Montpellier II, 34095 Montpellier Cedex 5, France.

The human pathogens *B. suis* S1 and *B. suis* S3 multiplied well within U-937, whereas the strains *B. canis*, *B. suis* S4 and IgG-opsonized *B. suis* S1 survived but did not multiply.As a control, *Escherichia coli* K12 was rapidly eliminated under the same conditions. Elimination was accompanied by a rapid and transient induction in TNFα production, and by a constant release of IL-6. Using IgG-opsonized *E. coli*, the same patterns of secretion but a stronger cytokine induction were observed, in parallel to enhanced bacterial killing immediately after infection. In contrast, all *Brucella* spp. strains used never induced TNFα secretion, whereas IL-6 secretion was strains-dependent. Rough *B. canis* induced a weak and constant IL-6 response in U-937, qualitatively similar to the results obtained with *E. coli*. On the other hand, smooth pathogenic *B. suis* S1 and *B. suis* S3 induced a weak but only transient IL-6 synthesis, accompanying bacterial multiplication. *B. suis* S4 did not induce any IL-6 production in the course of infection. These results will be discussed with respect to adenosine secretion, a process probably contributing to virulence of *B. abortus*.

B 303 CYTOKINE PRODUCTION BY HUMAN GINGIVAL FIBROBLASTS (HGF) CHALLENGED WITH PERIODONTOPATHOGENS. Jeffrey L. Ebersole, Michelle Steffen, Anna Dongari, and Stanley C. Holt, Dept. of Periodontics, U. of Texas Health Science Ctr. at San Antonio, TX 78284.

Periodontitis is a tissue destructive disease in the oral cavity which is clearly initiated by bacteria. Within the microbial ecology there exists a limited number of microorganisms that can exert pathogenic potential. It has also become evident that selected clinical parameters of this disease could result from local production of various pro-inflammatory and immune regulatory molecules by cells of the gingiva in response to bacterial colonization. HGF, comprising the gingival connective tissue, are critical cells in attachment of the supporting structures to the tooth surface. HGF produce an array of cytokines/mediators including: PGE_2, IL-1ß, IL-6 and IL-8. We have initiated extensive studies to profile these HGF products following challenge with the periodontopathogens: *A. actinomycetemcomitans, P. gingivalis, C. rectus,* and *T. denticola*. Studies of HGF challenge with these pathogens included: (i) kinetics and levels of production, (ii) dose responses of the bacterial challenge, (iii) live vs. dead bacteria, and (iv) environmentally stressed bacteria. These studies showed clear differences in the ability of different genera of oral pathogens to induce cytokines/mediators. In particular, *Aa* primarily elicited IL-1ß and IL-6. *Pg* stimulated PGE_2 and IL-6 while suppressing IL-8 production. *Cr* was the most potent inducer of IL-6, IL-8, and IL-1ß. *Td* elicited primarily PGE_2 and IL-1ß, with minimal activity towards the other cytokines/mediators. Dose response studies showed stimularoty levels of bacterial challenge, while increasing the bacterial numbers substantially down-regulated even the basal cytokine synthesis by HGF. Live and dead bacteria of the same species elicited quite different profiles of selected mediators (*eg. Pg;* PGE_2 and IL-6 ↑ with dead bacteria), while other cytokine/mediator production appeared similar irrespective of the viability of the bacteria. Finally, our findings showed that bacteria cultivated under environmental stress stimulated HGF to produce a different profile of mediators when compared to the same species grown in an enriched environment. Thus, **these studies are the first to detail relationships among cytokine/mediators produced by HGF in response to bacterial challenge. The resulting profiles were highly dependent upon the genera, amount, viability and surface characteristics of the bacteria. These findings are a requisite for understanding the host-bacterial relationships that occur in the gingival sulcus and contribute to the pathogenesis of the bacteria in this disease.**

B 304 EPITHELIAL CELLS SECRETE INTERLEUKIN-8 IN RESPONSE TO BACTERIAL ENTRY, Lars Eckmann, Joshua Fierer and Martin F. Kagnoff, Laboratory of Mucosal Immunology, Department of Medicine, University of California, San Diego, La Jolla, CA 92093

Bacterial invasion of mucosal surfaces results in a rapid influx of polymorphonuclear leukocytes. The chemotactic stimulus responsible for this response is not known. Since epithelial cells are among the first cells invaded by many enteric pathogens, we investigated the ability of epithelial cells to provide an early signal for the mucosal inflammatory response through the release of chemotactic cytokines. Monolayers of human epithelial cells (T84, Caco-2 and HeLa) were infected with various bacteria and, after 4 hours in culture, cytokine levels in the supernatants were determined by ELISA. We found that the chemokine interleukin-8, a potent chemoattractant and activator of polymorphonuclear leukocytes, was secreted by intestinal and cervical epithelial cells in response to bacterial entry. Moreover, a variety of different bacteria, including those that remain inside phagosomal vacuoles, e.g. *Salmonella dublin*, and those that invade the cytoplasm, e.g. *Listeria monocytogenes*, stimulated this response. Increased IL-8 mRNA levels could be detected within 90 min after infection. Neither bacterial lipopolysaccharide nor noninvasive bacteria, including *Escherichia coli*, *Enterococcus faecium* and an invasion-deficient mutant (*inv*A) of *Salmonella dublin*, induced an IL-8 response. Tumor necrosis factor alpha, which is known to be expressed by some epithelial cells, was not detected in the culture supernatants after bacterial entry. Moreover, addition of anti-tumor necrosis factor alpha antibodies had no effect on the IL-8 response following bacterial entry. These data suggest that epithelial cells serve as an early signalling system to host immune and inflammatory cells in the underlying mucosa following bacterial invasion. This work was supported by NIH grants DK35108 and DK40582, and a grant from the Universitywide AIDS Research Program.

B 305 INTERACTION OF CHLAMYDIA WITH THE HOST CELL SIGNAL TRANSDUCTION SYSTEM, Joanne N. Engel*, Farah Fawaz*, Chris van Ooij#, and Sarah Mutka†, Departments of Medicine*, Endocrinology#, and Biochemistry†, UCSF, San Francisco, CA 94143

The primary cell infected by chlamydia is the columnar epithelial cell found in the genital tract and in the conjunctiva of the eye. Subsequently, macrophages can be infected as well. Chlamydia must communicate with the normally non-phagocytic epithelial cells to induce its uptake. We have begun to examine this parasite-host cell communication by examining whether uptake of chlamydia by macrophages and epithelial cells is communicated via eukaryotic signal transduction pathways by looking for changes in host protein phosphorylation. First, we have performed immunoblot analysis of lysates from infected cells probed with antibodies to phosphotyrosine to detect changes in host protein tyrosine phosphorylation. Interestingly, within 15 minutes after infection and lasting for at least 18 hrs post infection of either epithelial cell lines or macrophage cell lines, we detect the increased tyrosine phosphorylation of a "family" of 80 kDa proteins using the antiphosphotyrosine immunoblot assay. These 80 kDa proteins are of host cell origin; we have failed to detect phosphotyrosine in purified EBs or RBs. We believe that the molecule responsible for inducing this phosphorylation event is closely associated with or is the chlamydial particle itself based on the following experiments. (i) it co-purifies with chlamydial elementary bodies through several density gradients (ii) it is not present in mock chlamydial stock made from uninfected cells (iii) it is not filterable away from chlamydial elementary bodies and (iv) the phosphorylation does not occur when the chlamydial elementary bodies are heat- or UV-inactivated (note that these EBs are taken up by the host cell but are degraded in lysosomes). The family of 80 kDa proteins appears to be partially cytoskeletal associated as approximately 50% of it is resistant to solubilization by 5% Triton-X 100 containing 1M NaCl. We are currently investigating whether the chlamydial-induced phosphorylation of these proteins is important to the intracellular survival of chlamydia or whether this is part of the host cell response to this foreign pathogen. In addition, we have utilized an *in vitro* kinase renaturation assay to detect changes in non-tyrosine protein kinases in chlamydial-infected cells. This assay has allowed the identification of several protein kinases, some host-encoded, and some parasite-encoded, whose activity changes during infection.

B 306 CHOLERA TOXIN B SUBUNIT-COATED MICROPARTICLES BIND SELECTIVELY TO PEYER'S PATCH M CELLS,
Andreas Frey, Hubert Reggio, Richard A. Weltzin, Wayne I. Lencer and Marian R. Neutra, Department of Pediatrics, Harvard Medical School, and GI Cell Biology, Children's Hospital, Boston, MA 02115.
M cells are specialized epithelial cells in the lymphoid follicle-associated epithelium of gut-associated lymphoid tissue, characterized by a large, basolateral pocket which hosts lymphoid cells and macrophages. M cells endocytose certain microbial pathogens that bind to their surfaces, and deliver them to the underlying antigen processing cells. This transepithelial transport process can be considered the initial step in the generation of a mucosal immune response. The molecular mechanism by which microbes adhere preferentially to M cells is not yet clear. While receptors unique to the M cell surface may mediate microbial attachment, it is also possible that increased accessibility of M cell surface components compared to villus absorptive enterocytes plays a crucial role.
This study was designed to test the hypothesis that certain ligands whose receptors are common to most intestinal epithelial cells can adhere preferentially to M cells when presented in particulate form. We used cholera toxin B subunit (CTB) as test ligand. Soluble, rhodamine-labeled CTB bound to plasma membranes of all epithelial cells, including M cells, via the ubiquitous ganglioside GM_1. In contrast, CTB-coated 15 nm colloidal gold particles, administered *in vivo* to rabbit Peyer's patches, adhered exclusively to M cells. This suggests that the gold particles had access to GM_1 on M cells but not on adjacent enterocytes. To examine whether CTB-coated particles in the size range of bacteria can still selectively adhere to plasma membrane GM_1 on M cells, we coupled CTB irreversibly to hydrophilic, fluorescent, 1μm latex microspheres without loss of CTB binding activity: CTB-modified microparticles bound specifically to GM_1-positive BALB/c 3T3 fibroblasts, and binding was blocked by competition with free, soluble CTB. Exposure of rabbit Peyer's patches *in vivo* to 5 x 10^8 CTB-microparticles resulted in strongly selective binding and uptake of microparticles in dome epithelium but not in adjacent villi.
We conclude that binding of CTB to GM_1 can be rendered M cell-specific by coupling to a particle of 1μm or less. These findings have important implications for the mechanism of microbial invasion via M cells, and for targeting of oral vaccines to the mucosal immune system.

B 307 ADHERENCE OF ENTERIC PATHOGENS TO M CELLS: ARE UNIQUE M CELL GLYCOCONJUGATES INVOLVED?,
Paul J. Giannasca[1], Per Falk[2], Jeffrey I. Gordon[2] and Marian R. Neutra[1], [1]G.I. Cell Biology, Children's Hospital and Harvard Medical School, Boston, MA 02115; [2]Dept. of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110
The epithelium overlying lymphoid follicles in the intestinal tract contains M cells specialized for transepithelial transport of antigens, a prerequisite for induction of a mucosal immune response. Various pathogens exploit the transcytotic pathway of M cells to gain entrance to the mucosa. The mechanisms by which pathogens recognize and adhere to the M cell apical surface and the M cell components that mediate this interaction are presently unknown. As a model for this initial step, we are examining the interaction between *Salmonella typhimurium* and M cells of Peyer's patches in mouse and rabbit. Incubation of wild-type *S. typhimurium* in ligated intestinal loops has revealed species differences in the effects of bacterial binding on M cells. Adherence and endocytosis of *Salmonella* by mouse M cells is accompanied by M cell damage, whereas rabbit M cells take up and transcytose the bacteria without obvious deleterious effects. Furthermore, a subpopulation of M cells is targetted by *Salmonella*: certain M cells bind multiple *Salmonella* while others bind none.
In an effort to identify M cell-specific surface components which may serve as recognition and binding sites for *Salmonella* and other bacteria, lectins were applied to paraffin and extracted Epon sections of mouse Peyer's patch tissue. Binding sites for a fucose-specific lectin (UEA I) were found in abundance on apical, basolateral and intracellular membranes of all Balb/c mouse M cells. Mucin in goblet cells and in the lumen also bound fucose lectin, but enterocytes on villi and in follicle-associated epithelium did not. A monoclonal antibody that recognizes blood group antigen H type 2 (a fucosylated glycoconjugate) stained the apical surfaces of some but not all M cells as well as certain goblet cells. Other fucose lectins (AAA, EEA, Lotus) labeled the apical surfaces of subpopulations of M cells. Thus, different fucose-bearing glycoconjugates appear to be expressed within the M cell population, revealing the existence of M cell subgroups. The possible role of M cell apical membrane glycoconjugates in the selective adherence of *Salmonella* and other enteric pathogens is under investigation.

B 308 VARIATION IN EXPRESSION OF THE VIRULENCE PHENOTYPE IN *Shigella sonnei* STRAINS: IMPLICATIONS FOR VACCINE DEVELOPMENT,
Antoinette B. Hartman and Malabi M. Venkatesan, Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC 20307
Construction of a stable *S. sonnei* vaccine strain has been complicated by the instability of the virulence phenotype caused by spontaneous loss of the large virulence plasmid. To select a suitable candidate for designing a vaccine strain, 16 *S. sonnei* strains were tested for stability of virulence expression. In some strains, such as 53G, 55-70% of the bacteria were Form I positive and HeLa cell invasive after overnight growth. Other strains, such as LB, showed greater stability with 85-95% of the bacteria remaining positive for both Form I expression and HeLa cell invasion after overnight growth. The growth patterns of 53G and LB were similar. The relative invasive ability as measured in a HeLa cell invasion assay indicated that the 53G strain is three-fold more invasive than strain LB. A streptomycin resistant derivative of LB, LBstr, and a growth deficient derivative, LB7, showed 8 to 10-fold lower invasion than the parent LB strain. A streptomycin resistant derivative of LB7, LB7str, has lost the ability to invade HeLa cells. While 53G and LB produced positive in Sereny reations, LBstr gave a delayed reduced response and LB7 was Sereny negative. When guinea pigs were immunized with LB7, 94% were protected against severe keratoconjunctivitis. In addition, variation in the restriction fragment hybridization patterns of the *ipaH* gene, a multicopy gene encoding IpaH, an invasion plasmid antigen, was observed in the 16 *S. sonnei* strains. Three *ipaH* hybridization patterns were observed, with the dominant form (type 1) present in 10 of the 16 strains examined (63%), while type 2 was observed in 5 of the 16 strains (31%) and type 3 in only 1 strain (6%). Additional characterization of these strains is ongoing.

B 309 OUTER MEMBRANE PROTEIN GENES rck AND ail ENCODE VIRULENCE-ASSOCIATED PHENOTYPES NOT ENCODED BY pagC, ompX OR lom.
Edwin J. Heffernan, Lydia Wu, Janice Louie, Sharon Okamoto, Joshua Fierer, and Don Guiney, Department of Medicine, University of California at San Diego, San Diego, CA 92120.
A five-member family of Enterobacteriacae outer membrane protein genes contains three genes with known virulence-associated phenotypes: rck, a S. typhimurium virulence plasmid serum resistance gene; ail, a Y. enterocolitica gene mediating epithelial cell attachment and invasion; and pagC, a S. typhimurium gene necessary for macrophage survival and virulence in mice. lom, expressed in lambda lysogens of E. coli, and ompX , a gene found in E. coli and E. cloacae, have no known functions. We compared the abilities of these genes to promote serum resistance and eukaryotic cell invasion in pBR322 constructs transformed into E. coli HB101. We find that both rck and ail constructs increase serum resistance (1.04 and 3.88 logs of kill, respectively) compared to pBR322 in E. coli HB101(> 6 logs of kill). We also compared bacterial invasion of eukaryotic cells by cell-associated survival in gentamicin and by examination of Giemsa-stained washed cells. Both ail and rck containing strains showed at least 90-fold greater cell-association than did controls (0.6% and 0.3%, respectively, vs. 0.0034%). In the latter assay, which does not distinguish between adherence and invasion, ail and rck show significantly greater association with CHO cells (6.3 and 0.9 bacteria per cell, respectively) and HepG2 human hepatocytes (1.2 and 1.8 bacteria per cell, respectively) than do controls (0.1 and 0.2 bacteria per cell for CHO and HepG2 cells, respectively). Neither pagC, ompX, nor lom constructs induce any increase in serum resistance or cell association compared to controls. ail and rck share the ability to encode for two distinct virulence-associated phenotypes not expressed by the other members of the gene family.

B 310 ***SHIGELLA FLEXNERI*** **CHALLENGE OF HELA CELLS ALTERS TRANSCRIPTION FACTOR DNA BINDING ACTIVITIES**, Norbert K. Herzog, Roy B. Dyer, Christopher Collaco, and David W. Niesel, Departments of Microbiology and Pathology, University of Texas Medical Branch, Galveston, TX 77555

Although information about the genetic basis and mechanisms of *Shigella flexneri* cellular invasion is accumulating, little is known about changes in cell signalling and their consequences following bacterium-host cell interactions. A general consequence of signal transduction is alterations in the levels and/or activities of transcription factors (TFs). DNA-binding activities of cellular TFs to AP1, AP2, cyclic AMP responsive element, CTF/NF1, NF-κB/*rel*, OCT1, and SP1 DNA binding sites were altered following *S. flexneri* challenge as measured by electrophoretic mobility shift assays (EMSA). NF-κB/*rel* DNA-binding activity was enhanced more than 11-fold by cellular invasion; noninvasive *S. flexneri* strains induced only low activity levels of NF-κB/rel. Both p50 and p65 but not c-rel were found to be components of the κB DNA-binding activity. These data suggest that changes in cellular transcription factor binding activity are a consequence of *S. flexneri* invasion. Other experiments have shown alterations in TF DNA-binding activities as a consequence of challenge by other pathogenic bacteria. Alterations in TF DNA-binding activities could be a general mechanism in the initial host response or in the pathogenesis of these diseases.

B 311 IgA1 PROTEASES IN THE PATHOGENESIS OF INVASIVE INFECTIONS: EVIDENCE IN SUPPORT OF A HYPOTHETICAL MODEL, Mogens Kilian and Jesper Reinholdt. Institute of Medical Microbiology and Department of Oral Biology, University of Aarhus, The Bartholin Building, 8000 Aarhus C, Denmark.

By convergent evolution the three principal causes of bacterial meningitis, gonococci, and certain other bacteria that colonize mucosal surfaces have developed the ability to cleave the hinge region of human IgA1 at one of several post-proline peptide bonds. IgA1 accounts for more than 90% of IgA secreted into the rhinopharynx. Cleavage by IgA1 proteases efficiently eliminates secondary effector functions of S-IgA1 antibodies including their ability to inhibit microbial adherence, to agglutinate, and to interact with the mucus layer covering mucosal epithelia. The released monomeric Fab fragments retain antigen-binding activity. IgA1 protease-producing bacteria growing in the presence of specific IgA1 antibodies become coated with Fab_α fragments which block access of intact antibody molecules and inhibit their potential bactericidal activity. Acquisition of *H. influenzae* type b, *N. meningitidis*, or *S. pneumoniae* and the subsequent temporary nasopharyngeal colonization induce mucosal antibodies to both surface antigens and IgA1 protease of the potential pathogen resulting in immunity. We have hypothesized that susceptibility of occasional individuals to invasive disease is the result of preexisting IgA1 antibodies in secretions and sera against surface antigens of the pathogen. These antibodies are induced by prior mucosal contact with cross-reacting microorganisms and, therefore, do not include inhibiting antibodies to the IgA1 protease of the pathogen. As a result, the pathogen is able to cover itself by impotent Fab_α fragments, and the resulting enhanced adherence and efficient evasion of the immune system may allow the bacteria to penetrate the mucosal barrier and cause systemic infection. In support of this hypothesis we have demonstrated cross-reactive bacteria in the gut of 52% (N=69) of children hospitalized with *H. influenzae* type b meningitis and epiglottitis. The prevalence in age-matched controls was < 2%. These findings indirectly support the hypothesis that IgA1 proteases play an important role in some invasive bacterial infections.

B 312 SALMONELLA-MONOCYTE INTERACTION IN REACTIVE ARTHRITIS. Juha Kirveskari, Marko Salmi, Juhani Tuominen, Sirpa Jalkanen, Kaisa Granfors. National Public Health Institute, Department in Turku and Department of Biostatistics, University of Turku, SF-20520 Turku, Finland.

Persistence of bacterial structures in the body for long time after acute infection seems to have an important role in the pathogenesis of reactive arthritis after Salmonella, Shigella and Yersinia infections. Lipopolysaccharide structures have been detected also in inflamed joints, within mononuclear and polymorphonuclear phagocytes. It seems likely, that the interaction between host and microbe is abnormal and inefficient in HLA B27 positive subjects in whom reactive arthritis develops.

In order to study the interaction of Salmonella bacteria with mononuclear phagocytes we fed peripheral blood monocytes of healthy HLA B27 positive and negative persons with live Salmonella enteritidis bacteria, and followed the expression of certain cell surface antigens during 7 days of incubation. Despite Salmonella bacteria were living intracellularly, also obvious procession of bacteria started in few hours leading to expression of Salmonella antigens on the cell surface. Otherwise on the cell surface, CD14 was first downregulated, and then markedly upregulated. Expression of common β_1-chain was regulated in the same fashion. CD11a, CD11b and CD11c were all first downregulated, the response was most rapid in CD11b. However, baseline expression of CD11c increased later. Expression of MHC class II antigens DR, DP, DQ was not much affected in the beginning of procession, but was slightly downregulated at 5 to 7 days of incubation.

Salmonella bacteria inside the monocytes enhanced the adhesion of these cells to endothelial cell monolayers, probably by modulating the expression of CD14. Enhanced adhesion may contribute to increased transmigration of these cells and a subsequent inflammatory response.

B 313 **Cholera toxin and *Salmonella* induce production of different cytokines in the gastrointestinal tract.** **Gary R. Klimpel and David W. Niesel, Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77555.**

Cholera toxin (CT) and *Salmonella* are potent inducers of fluid secretion in the gastrointestinal tract. Exposure of the gastrointestinal tract to CT does not result in pathology or inflammation. In contrast, *Salmonella* infection of the gastrointestinal tract results in inflammation and pathological changes in the infected tissue. We have recently shown that TNFα mediates the early pathology in *Salmonella* infection of the gastrointestinal tract. Using a mouse ligated intestinal loop model, we extended this study by investigating cytokine production in intestinal loops challenged with CT versus *S. typhimurium* (TML). Fluids from intestinal loops were assessed for IL1, IL2, IL4, IL6, IL10, INFγ and TNFα using bioassays and ELISA. Elevated levels of cytokine mRNA were assessed by RT-PCR. Ligated loops challenged with *Salmonella* were shown to produce high levels of IL1α, IL6, IL10, IFNδ and TNFα. IL2 and IL4 production were not detectable in *Salmonella* challenged loops. In contrast, CT challenged loops only produced IL10 and IL6. These results suggest that the lack of intestinal pathology associated with CT exposure maybe due to the regulation of pro-inflammatory cytokine production by CT/IL10.

B 314 PASSAGE OF *SALMONELLA TYPHI* THROUGH CACO-2_{BBe} CELL MONOLAYERS DIFFERS FROM THAT OF *S.TYPHIMURIUM*, Sandra K. Kops, Michael G. Kashgarian, William H. Marks, and A. Brian West, Yale University Department of Pathology, New Haven, CT 06510.

Virulent *Salmonella typhi* of strain Ty2 traverse mature confluent monolayers of CACO-2_{BBe} cells on Transwell filters within 15 minutes. Transmigration is marked by a rapid decline in transepithelial electrical resistance and by increased permeability to ^{3}H-mannitol, and is actin-dependent as shown by 98% inhibition by cytochalasin B.

Like *S. typhimurium* (Finlay and Falkow, J Infect Dis 1990;162:1062), *S. typhi* causes severe disturbance to the brush border and blebbing of the apical cytoplasm. However, while we observed numerous *S. typhimurium* within CACO-2_{BBe} cell endosomes by electron microscopy, *S. typhi* were infrequently seen within the cytoplasm of the epithelial cells. Immuno-electron microscopy using antibodies directed against H and O antigens showed staining of the cell wall and fimbriae of extracellular bacteria. Close to the brush border, some *S. typhi* were unstained, as were the few intracellular bacteria observed, though unstained *S. typhimurium* were not seen. Membrane-bound aggregates of amorphous material found in cells exposed to *S. typhi*, but not in those exposed to *S. typhimurium* or in bacteria-free controls, were immunoreactive with the anti-H antibody, and are therefore likely to be of bacterial origin. *S. typhi* caused increased apoptosis and mitotic activity in the monolayers, features suggesting a cytopathic effect which were not noted with *S. typhimurium*.

These observations indicate that *S. typhi* causes more severe injury to epithelial cell monolayers than does *S. typhimurium*, that it may not replicate intracellularly, and that passage through the monolayer is associated with loss of H and O antigens. *S. typhi* may traverse enterocyte monolayers by a mechanism different from that described for *S. typhimurium*.

B 315 ANTIGENIC HETEROGENEITY AMONG *STREPTOCOCCUS PNEUMONIAE* IgA1 PROTEASES. Hans Lomholt, Uffe B. Skov Sørensen, and Mogens Kilian, Institute of Medical Microbiology, University of Aarhus, and Statens Serum Institut, Copenhagen, Denmark. *Streptococcus pneumoniae* produce a protease highly specific for the proline-threonine bond at position 227-228 in the hinge region of the human immunoglobulin A1 heavy chain (IgA1 protease). IgA1 is the principal immunoglobulin class protecting mucosal surfaces of the upper airways and cleavage results in intact antigen binding Fab_α fragments devoid of the Fc_α effector domains. If a colonized individual responds with antibodies to bacterial surface constituents but not with antibodies that neutralize the IgA1 protease, then the pathogen is potentially able to shield itself by binding Fab_α antibody fragments. The resulting evasion of the immune system may contribute to bacterial invasion of the mucosal barrier.

In this study enzyme neutralization assays were used to examine epitopes on *S. pneumoniae* IgA1 protease. Between 7 and 11 strains from each of nine serotypes most frequently causing childhood infections in Denmark (1, 3, 6A, 6B, 7F, 14, 18C, 19F, 23F) were examined for inhibition by 9 rabbit antibody preparations raised against selected pneumococcal IgA1 proteases. Twenty five strains representing different combinations of IgA1 protease inhibition types and serotypes were further characterized by 7 additional antibody preparations. For each reaction the titer of inhibition was determined. At least 15 IgA1 protease inhibition types were discriminated with relatively limited cross-reactions between types. Isolates of some serotypes appeared homogeneous in producing IgA1 proteases of the same inhibition type (serotypes 3 and 7F) whereas isolates of other serotypes were more heterogeneous producing IgA1 proteases of up to four different inhibition types (18C and 19F). No clear correlation was found between IgA1 protease inhibition type and serotype as some inhibition types were found among strains of different serotypes. In conclusion, *S. pneumoniae* IgA1 proteases display a remarkable heterogeneity in epitopes recognized by neutralizing antibodies.

B 316 EXPRESSION OF ADHERENCE, INVASION AND VIRULENCE OF *SALMONELLA TYPHIMURIUM* AS A FUNCTION OF OSMOLARITY AND TEMPERATURE, D. Scott McVey, M.M. Chengappa and Nathan H. Gabbert, Department of Pathology and Microbiology and Department of Clinical Sciences, Kansas State University, College of Veterinary Medicine, Manhattan, KS 66506

The majority of racing greyhound diets contain significant portions of raw meat. This meat is contaminated with numerous microorganisms, including salmonellae. Further, many veterinarians have observed that feeding stored meet is associated with a higher incidence of gastroenteritis caused by *Salmonella* (*S.*) *typhimurium*. In numerous recent studies the pathogenesis of salmonellosis has been more clearly defined at the molecular level. Although much work remains to be completed, it is clear that environmental conditions are important regulators of expression of virulence factors. These virulence factors include adherence to and invasion of intestinal cells. The purpose of the experiments presented here was to characterize the expression of these virulence properties of *S. typhimurium* as a function of osmolarity, temperature and growth phase. The temperatures selected represent common holding temperatures for raw meat. The *S. typhimurium* isolate was obtained from greyhounds with enteric salmonellosis. Bacteria were cultured in Luria-Bertani broth medium with addition of either .05, .20 or .35M NaCl. These cultures were incubated at 22°, 30° or 38° C in glass tubes with moderate agitation. Similar to results obtained by other investigators from other enterobacteriaceae, increased osmolarity did significantly increase both adherence and invasion of Henle 407, Caco-2 and MDCK cells. This increase in adherence and invasion was observed at each of the incubation temperatures. Bacteria from cultures that entered stationary phase were generally less adherent (0 to 5 % adherent) than bacteria from log-phase cultures (22 to 35% adherent). However, bacteria that were incubated at 22° C in LB broth with .35 M NaCL retained the ability to adhere to and invade MDCK cells even through early stationary phase. These findings were reflected in in vivo virulence studies in weanling BALB/c mice. Low-temperature (22° C), stationary-phase cultures of the same *S. typhimurium* were more virulent (oral $LD_{50}=10^4$) than cultures from higher temperature cultures (30° and 38° C, $LD_{50}=5 \times 10^5$). These data indicate that *S. typhimurium* from some ambient temperature sources may be more virulent and possibly more dangerous as food contaminants. As the general knowledge with regard to canine salmonellosis increases, more specific measures can be constructed and/or improved to reduce illness and infection in greyhound dogs.

B 317 ***SHIGELLA FLEXNERI*-HELA CELL INTERACTIONS: A PUTATIVE ROLE FOR HOST CELL PROTEIN KINASES,** David W. Niesel, Christopher Collaco, Roy B. Dyer, and Norbert K. Herzog, Departments of Microbiology and Pathology, University of Texas Medical Branch, Galveston, TX 77555

Epithelial cell invasion has been shown to be prerequisite for *Shigella flexneri* virulence. While components of the bacterial cell surface and alterations in the epithelial cytoskeleton are involved in this process, little is known about the putative cell signalling events. Recently, we have established the induction of transcription factor DNA binding activities as a result of *S. flexneri* challenge of HeLa cells. The other components of this cell signalling pathway are presently unknown. HeLa cells challenged with *S. flexneri* were shown to possess differences in phosphotyrosine containing proteins. These changes were detected as early as 5 min. post-challenge. Challenge with a noninvasive *ipa*B mutant strain resulted in the induction of an altered profile of phosphotyrosine containing host cell proteins. Phosphotyrosine containing proteins could be detected in *S. flexneri*, but were unique from those detected following HeLa cell challenge. *S. flexneri* invasion of HeLa cell monolayers was reduced following treatment with protein kinase inhibitors (staurosporine, 50% at 10 μm; genistein, 25% at 100 μm) prior to and during bacterial challenge. Interestingly, the serine/threonine protein kinase inhibitors H7 or H8 were found to enhance bacterial uptake after short treatment times. The effects of treatment with combinations of H7 and H8 were found to be additive. These data suggest a role for protein kinases in the initial response of host cells to *S. flexneri*.

B 318 PULMONARY ANTIBODY AND CYTOKINE PRODUCTION IN A MOUSE LUNG SHIGELLA INFECTION MODEL. Lillian L. Van De Verg, Hugh H. Collins, Corey P. Mallett and Thomas L. Hale, Walter Reed Army Institute of Research, Wash. DC. 20307

Mice inoculated with pathogenic Shigella via the intranasal route develop localized inflammatory lesions in the lungs as a result of bacterial invasion of mucosal epithelial cells, followed by intercellular spread. The development of localized lesions resembles that seen in human colonic epithelium during Shigella dysentery. In this study, the mouse lung infection model was used to examine the profile of local antibodies in immune mice. Differences between immunized and susceptible mice in terms of pulmonary cytokine levels after lethal (LD_{70}) challenge with Shigella flexneri 2a were also studied. Immune mice were vaccinated with two sublethal doses of wild type S. flexneri 2a and subsequently developed high levels of secretory IgG and IgA antibodies against both lipopolysaccharide (LPS) and invasion plasmid antigens (Ipa). Compared to susceptible mice, immune mice had significantly higher levels of pulmonary gamma interferon (G-Ifn) as early as 6 hours following challenge; levels peaked at 24 hours for both groups and were still detectable at 48 hours. Tumor necrosis factor (TNF) levels were detected in both immune and susceptible mice at 6 hours; at 48 hours, TNF levels were significantly higher in susceptible mice. Small amounts of TNF were detected in immune mice sera at 6 hours; otherwise sera were negative for TNF and G-Ifn. Interleukin-4 levels were only found in the lung lavage and sera of immune mice at 48 hours. These results are consistent with our findings that there is an early influx of CD4+ cells and macrophages after challenge, followed by the appearance of antibody secreting cells at around 72 hours. Secretory antibody and local cytokines may both play a role in the early clearance of bacteria we have noted in immune mice.

B 319 AN INVASION PLASMID-DERIVED *SHIGELLA FLEXNERI* GENE WITH HOMOLOGY TO IS*629* AND TO BACTERIAL SUGAR PHOSPHATE TRANSPORT PROTEIN SEQUENCES. Malabi M. Venkatesan, Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The invasion of epithelial cells in tissue culture by *Shigella* is associated with the presence of a 220 kb plasmid found in all virulent strains. Transposon mutagenesis and subcloning experiments has shown that a 40 kb region on this invasion plasmid is sufficient to confer the invasive phenotype to an *E. coli* K12 strain. The *ipaBCDAR* genes and the spa genes define the two margins of this 40 kb segment. Two of the five plasmid copies of the *ipaH* genes in *S. flexneri* 5 (strain M9OT-W, plasmid pWR100) are present in a head-to-tail arrangement approximately 8 kb upstream of *ipaR*, outside the 40 kb invasion-associated region. Sequences flanking the $ipaH_{4.5}$ gene were found to give multiple bands with both pWR100 and the M9OT-W chromosome. DNA sequence analysis of 2684 bp immediately downstream of the TGA stop codon of $ipaH_{4.5}$ indicate that the initial 505 bp contained the first ORF of IS629 (same as IS3411) and the beginning of a second ORF which is presumed to encode the IS629 transposase. The first 27 amino acids of the transposase ORF is fused to a protein sequence giving a total length of 335 amino acids, that shows significant homology to *E. coli* and *S. typhimurium* UhpT proteins, which catalyzes the active transport of hexose phosphate, and to *S. typhimurium* PgtP and GlpT proteins which are involved in the uptake of phosphoglycerate (2-phosphoglycerate, 3-phosphoglycerate and phosphoenol pyruvate) and sn-glycerol 3-phosphate respectively. The uhp, pgt and glp systems for sugar phosphate uptake are each composed of three to four genes one of which is a transporter protein (*uhpT*, *pgtP* & *glpT* genes) and are induced only in the presence of extracellular sugar phosphates. The three transporter proteins show significant homology to each other and also to the M9OT-W gene that is being referred to as the invasion plasmid gene *ipgD*. The sequence of *ipgD* that corresponds to the sugar phosphate transport encoding region is present as a single copy only on the invasion plasmid while the IS*629* portion of *ipgD* gene hybridizes to several bands on the plasmid and chromosome. An insertion mutant in the *Shigella ipgD* gene is currently being tested to determine its role in Hela cell invasion, plaque formation and keratoconjunctivitis in guinea pigs. Hybridization experiments with PCR-derived IS*600*, IS*629* and IS*630* probes indicate that there are 2-3 IS sequences that flank the M9OT-W 40 kb invasion-associated loci on either side. DNA sequencing of these regions are being carried out to confirm their extent, exact map location and orientation with respect to each other.

B 320 THE PATHOGENIC POTENTIAL OF A ß-HEMOLYSIN FROM SERPULINA HYODYSENTERIAE . Michael J. Wannemuehler, Dhuha Akili, David Hutto, Kristi Harkins, Malcolm Crump, and Mary Jo Schmerr, Departments of Micobiology, Immunology and Preventive Medicine, Veterinary Physiology and Pharmacology, Iowa State University, and The National Animal Disease Center, Ames, IA 50011.

Swine dysentery, caused by *Serpulina hyodysenteriae*, is a mucohemorrhagic diarrheal disease. Histologic lesions include mucosal erosions, inflammatory cell infiltration of the lamina propria, and coagulative necrosis of the superficial mucosa. Pathogenicity has been associated with colonization of the colon and production of a ß-hemolysin. Using ion exchange chromatography and HPLC gel filtration, a fraction containing the hemolytic activity was identified (M_r between 19 and 23 kDa). When the ß-hemolysin was added to a monolayer of colonic epithelial (Caco-2) cells, membrane integrity was diminished as measured by a reduction in the electrical resistance of the monolayer. The incremental addition of hemolysin to the monolayer was more effective in inducing changes in resistance than a single dose of hemolysin. The effect of the hemolysin appeared to be transient since cellular or monolayer integrity recovered within 4 to 6 hours as determined by an increase in the electrical resistance. Using indo-1 in a flow cytometric assay, it was demonstrated that the ß-hemolysin induced an increase in intracellular calcium concentrations in Caco-2 cells as well as peripheral blood lymphocytes (porcine or human). In similarly treated cells, there was no detectable increase in intracellular manganese suggesting that the hemolysin specifically affected calcium uptake. The hemolysin was also shown to enhance the cytotoxic effects of TNF for L929 cells. It was shown that 1 to 10 units of the hemolysin alone augmented growth of L929 cells; however, if 10 units of hemolysin was added to a non-lethal dose of TNF (0.008 units), cell death was potentiated. There appeared to be a synergistic effect between the ß-hemolysin and TNF. The mechanism(s) by which this beta-hemolysin participates in pathogenesis may include an alteration in membrane function or integrity as well as metabolic changes mediated by an increase in intracellular calcium.

B 321 REGULATION OF GENES NECESSARY FOR INVASION OF SHIGELLA SONNEI BY ENVIRONMENTAL pH AND ITS POSSIBLE SIGNIFICANCE IN VIVO. Haruo Watanabe, Jun Terajima, Syu-ichi Nakamura, and Tomoko Murai. Department of Bacteriology, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan

Genes spanning about 27-kb region of S. sonnei form I invasion plasmid were required for bacterial cell invasion. IpaBCD gene products, which are thought to be invasins of Shigella, are secreted to the surface of bacterial outer membrane and their expression is regulated by dual positive regulators virF and invE. Invasiveness of Shigella is regulated by environmental conditions, temperature and osmolarity. In this paper, we described that invasiveness of Shigella and E. coli K-12 carrying form I invasion plasmid was also controlled by external pH. Cell invasion of these strains was repressed under the pH of 6.4. This repression occurred in the expression of a positive regulator virF gene at the transcriptional level; the expression of virF-regulated genes, ipaBCD, virG invE, etc. was reduced when the bacteria were cultured in the low pH-buffered medium. The virF gene's repression was partially supressed by hns mutation. We isolated a Tn10-insertion mutant different from hns, in which virF expression and cell invasiveness were greater at low pH(pH6.0) than at high pH(pH7.4). This mutation did not affect the regulation by temperature and osmolarity. What is the significance of pH regulation for invasion gene's expression ? Antigen presentation into the MHC class II is thought to be occurred in the phagosome under the low pH conditions. If invasion genes are not expressed in the phagosome (low pH), Shigella may not efficiently present its own antigens into the host and escape from host defence.

Developmental Changes that Occur in Vivo

B 400 THE BVG CONTROLLED MOTILITY REGULON OF *BORDETELLA*. Brian Akerley and Jeff F. Miller, UCLA School of Medicine, Los Angeles, CA 90024-1747.
The *bvgAS* locus mediates a biphasic transition in *Bordetella spp.*by activating the majority of known virulence factors and simultaneously exerting negative control over a class of genes called *vrg*'s (*bvg*-repressed loci). Our previous results showed that BvgAS negatively controls the production of flagella and motility in *B.bronchiseptica*. Further analysis of the motility phenotype of *B.bronchiseptica* suggested that the *Bordetella* virulence control system mediates transcriptional regulation of a flagellar hierarchy that includes a regulatory locus, *frl*, which can complement *E. coli flhDC* mutants, an alternative sigma factor, and the *flaA* gene encoding flagellin. The *frlAB* locus was cloned and characterized to extend this study of negative control by BvgAS. The *B. bronchiseptica frlAB* exhibited extensive amino acid similarity to the *flhDC* operon of *E. coli*. Analysis of *frl* mRNA indicated that *frl* is negatively regulated by BvgAS at the level of transcription. An insertion mutation in *frlB* demonstrated that this locus is required for motility in *B. bronchiseptica*. A genetic screen for de-repression of *frl* transcription using a transcriptional fusion of *frl* to the chloramphenicol acetyltransferase gene suggested that BvgAS may represent the only negative regulator of *frl* expression. A *frl* probe detected hybridizing bands on genomic Southern blots of *B. pertussis*. The *frlAB* of *B. pertussis* strain BP536 was cloned and found to complement a *B. bronchiseptica frlB* mutant and *E. coli flhD* and *flhC* mutants. Analysis of the 5' region of the *B. pertussis frl* allele detected several nucleotide substitutions when compared with *B. bronchiseptica frl*. To date, motility control represents the only known phenotype associated with *flhDC* analogues. The discovery of a functional *frl* locus in the non-motile *B. pertussis* species raises the possibility that this locus may participate in the control of phenotypes other than motility. The selective loss and retention of phenotypes exhibited by these highly related members of the *Bordetella* genus may reflect differences in host specificity and the selective pressures encountered by these bacterial pathogens.

B 401 **Transcription of the Toxin Coregulated Pilus operon and the role of the *tcpA* promoter in ToxR/ToxT global regulation of virulence factors in *Vibrio cholerae*.** R. Clark Brown and Ronald K. Taylor, Depart. of Microbiology and Immunology, University of Tennessee, Memphis. TN 38163

ToxR is a transmembrane protein involved in the coordinate expression of several virulence genes including those coding for the Toxin Coregulated Pilus (TCP). The expression of the pilin gene, *tcpA*, is dependent on ToxR and on the *toxT* gene located downstream in the TCP gene cluster. ToxT's role is based on its ability to activate a *tcpA*-Tn*phoA* fusion when cloned in *E. coli*. In order to clarify the molecular mechanisms by which ToxR and ToxT control TCP expression, the *tcpA* promoter was examined for its contribution to the transcription of the downstream accessory genes, including *toxT*, and for its ability to be directly activated by both ToxR and ToxT. As expected, the influence of ToxR was found to be at the level of transcription using RNA analysis and *tcp* transcriptional fusions in *V. cholerae*. A single 800bp *tcpA* message was identified through northern analysis that correlates by size to the distance between the transcriptional start determined by primer extension and the hairpin loop sequence between *tcpA* and *tcpB*. However, RNase protection analysis demonstrated an additional *tcpAB* cotranscript as well as the dependency of the transcription of the *tcpC-tcpR-tcpD-tcpS-tcpT-tcpE-tcpF-toxT-tcpJ* region on *tcpA* transcription. Genetically, a polar *tcpA*-Tn*phoA* fusion could not be complemented for pilus surface expression by providing *tcpA in trans*. Thus, all evidence suggests that the *tcp* genes, including *toxT*, are organized in an operon dependent on ToxR, and that the majority of *toxT* expression is from the *tcpA* promoter. Operon fusions demonstrated that transcriptional activation can be accomplished by ToxT in *E. coli*. While, ToxR, ToxR-ToxS, or ToxR-PhoA-S failed to activate the *tcp* operon fusion in *E. coli* and a ToxR extract failed to bind to the *tcpA* promoter region in DNA mobility shift assays. Further investigation of how *tcp*-Tn*phoA* insertions polar on *toxT* expression can be regulated showed that a low basal level of *toxT* message was present in *toxR* and *tcpA*-Tn*phoA* strains grown in ToxR expressive or repressive conditions. These observations suggest that even if ToxT is autoregulatory via the *tcpA* promoter, activation of the polar fusions should not occur unless ToxT's potential is influenced by ToxR in a manner different from ToxR's role in exotoxin expression, or there is a weakly ToxR-activated promoter downstream of all previously identified *tcp*-Tn*phoA* fusions affecting the level of ToxT. Models on how ToxR might influence ToxT and how this impacts on the activation of other virulence genes are proposed.

B 402 *THE NORMAL INTESTINAL MICROFLORA AND ITS* EFFECTS ON GLYCOSYLATION, GENE INDUCTION AND CELLULAR PROLIFERATION IN THE EPITHELIUM OF THE SMALL INTESTINE, Lynn Bry, Per Falk, Tore Midtvedt*, Jeffrey I. Gordon, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, *Department of Medical Microbial Ecology, Karolinska Institute, Stockholm, SWEDEN

We have investigated the effects of the normal gut microflora on the development and proliferation of the small intestinal epithelium in a model system using NMR1 mice. These mice have been maintained in one of four environments, germ free, ex-germ free, conventional and monocontaminated with the mucin degrading strain, *Peptostreptococcus micros*. In each case we have used a variety of mRNA, antibody and lectin probes to assess the effects of these alterations on the intestinal epithelium. Lectin probes including *Ulex europaeus* I and AAA (*Anguilla anguilla*) reveal substantial differences in the patterns of glycosylation among the germ free, ex-germ free, conventional and monocontaminated small bowels. These differences become apparent only during and after the weaning period, suggesting that factors other than changes in the microbiota, e.g. hormonal and dietary factors, may be involved as well. The changes include a loss of fucose epitopes in the the goblet cell lineage in germ free animals while conventional and ex-germ free animals acquire staining in the enterocytic lineage. Monocontaminated animals demonstrate a partial activity in the enterocytic lineage. We believe that these alterations arise from the transcriptional induction of novel glycosyltransferases. We have also noted perturbations in the levels of cellular proliferation in germ free and conventional animals as detected by the labeling of actively dividing cells with 5'-Bromo-2'-deoxyuridine. These perturbations extend throughout the GI tract but do not become significant until after the first week of life. In conclusion, we have detected a variety of changes in the intestinal epithelium induced exclusively by the presence of the normal intestinal microflora. We believe that these changes may have an effect on establishing and on maintaining the mucosal barrier functions that play a significant role in preventing colonization and invasion by pathogenic microorganisms.

B 403 USE OF GENETIC RECOMBINATION TO REPORT *IN VIVO* VIRULENCE GENE EXPRESSION, Andrew Camilli, David T. Beattie and John J. Mekalanos, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

We have developed a gene expression reporter approach which uses the products of genetic recombination to serve as heritable reporters of prior gene expression. Induction of virulence gene transcriptional fusions made to the Tn$\gamma\delta$ resolvase gene, *tnpR*, results in the resolution (excision) of an artificial cointegrate substrate consisting of a tetracycline-resistance marker flanked by the resolvase target DNA sequences (*res* sequences). Loss of tetracycline-resistance in descendant bacteria serves as a permanent and heritable marker of prior gene expression. The induction of gene expression can be assayed at a later time and/or different place from the inducing environment facilitating the screening for, and study of, pathogen gene expression in host tissues and/or within tissue culture models of infection.

B 404 A RABBIT MODEL FOR THE ANALYSIS OF VIRULENCE GENE REGULATION IN *BORDETELLA BRONCHISEPTICA*, Peggy A. Cotter and Jeff F. Miller, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024-1747.
Expression of a majority of the known virulence factors synthesized by *B. pertussis*, the causative agent of whooping cough, is regulated by the BvgAS signal transduction system. BvgAS mediates a switch between two distinct phenotypic phases, the Bvg+ phase in which adhesins and toxins are expressed, and the Bvg- phase in which Bvg repressed loci are expressed. Our goal was to develop a natural *Bordetella*-host animal model to investigate the role of BvgAS mediated signal transduction *in vivo*. Since *B. pertussis* has adapted exclusively to the human host we chose to study the interaction between the closely related organism *Bordetella bronchiseptica* and one of its natural hosts, the rabbit. The *B. bronchiseptica* and *B. pertussis* BvgAS sensory transduction systems are 96% identical at the amino acid level and function analagously to regulate expression of virulence factors in response to environmental signals. Our first assessment of the model was a study of 4-month old New Zealand White rabbits with naturally acquired *B. bronchiseptica* infections. The wild type strain RB50 was isolated from the nares of these rabbits and the course of infection was followed for five weeks. To investigate the role of BvgAS regulation we constructed Bvg^C (RB53) and Bvg- (RB54) phase locked derivatives of RB50. RB53 contains a single bp mutation in *bvgS* causing a His to Arg change at position 570 in the linker region of BvgS resulting in constitutively active protein. RB54 contains an in-frame deletion in *bvgS* which spans the regions encoding the linker, transmitter and receiver domains. RB50 and RB53, but not RB54, were able to establish respiratory infections in *Bordetella*-free rabbits with an intranasal ID_{50} of less than 200 organisms. Three weeks after inoculation bacteria were recovered from the nasal turbinates, larynx, trachea and lungs in equivalent numbers. Histological examination of lung sections revealed no gross pathology in either wild type or mutant infected rabbits. Western blot analysis indicated that antibodies were generated against several Bvg+ phase specific antigens as well as proteins common to both phases. Anti-whole cell and anti-FHA titers rose 1000 fold during the infection as determined by ELISAs. *Bordetella*-specific antibodies directed at surface exposed antigens were detected by Immunoelectronmicroscopy and Immunofluorescent antibody staining. Parameters associated with initial colonization and establishment of infection by RB50 and RB53 were indistinguishable, suggesting a subtle role for BvgAS mediated signal transduction in the *Bordetella*-host interaction.

B 405 INSERTION OF TN*PHOA* IN THE PHO REGULON OF A SEPTICEMIC *ESCHERICHIA COLI* O115: K"V165":F165 PRODUCES AN AVIRULENT MUTANT, France Daigle, Josée Harel and John M. Fairbrother, Department of Pathology and Microbiology, University of Montreal, St-Hyacinthe, Québec, Canada
Escherichia coli O115:K"V165":F165 strains have been associated with septicemia in calves and piglets and are serum resistant. Using Tn*phoA* transposon mutagenesis in one of these strains, 5131, we obtained a *phoA* active mutant which was serum sensitive, avirulent in 1-day-old chicks and had lower total carbohydrate content. On transmission electron microscopy, the mutant showed a reduced amount of surface polysaccharides compared to the parent strain. By physical mapping with the Kohara bank, the insertion was located at 83 min on the chromosome of *E. coli*. The Tn*phoA* insertion with flanking sequences was cloned and sequenced. The insertion was located in the *pstC* gene which codes for a hydrophobic protein that forms the transmembrane portion of the Pst system. Pst system belongs in a class of periplasmic permeases and is regulated by the Pho regulon. The complemented mutant showed a total carbohydrate content higher than the mutant and became serum resistant. The *pst* mutation is pleitropic and our results demonstrated that it could have an effect on the production of the surface polysaccharides of strain 5131. Such mutations may give insight into new approaches to develop attenuated *E. coli* strains useful for vaccine strategies.

B 406 MACROPHAGE INDUCED PROTEIN SYNTHESIS IN *BRUCELLA ABORTUS* AND COMPARISON WITH HEAT SHOCK, ACID SHOCK AND OXIDATIVE STRESS INDUCED EXPRESSION. Thomas A. Ficht, Goubin Song and Jhyshiun Lin. Department of Veterinary Pathobiology, Texas A&M University and Texas Agricultural Experiment Station, College Station, Texas 77843.

Survival of *Brucella* within macrophages has been shown to correlate with infection and disease. Following phagocytosis, *Brucella* confront at least two different environments; (i) the phagosome, a physiologic pH environment, and (ii) the phagolysosome, a low pH and oxidative environment. The ability of *Brucella* to survive depends upon gene expression in response to these environments. We have characterized protein synthesis in *B. abortus* following macrophage phagocytosis and in vitro enviromental stress using one- and two-dimensional gel electrophoresis. Polypeptides which quantitatively increased during various stress were cataloged. Although overlap was noted, only a few proteins induced by macrophage phagocytosis were also identified as acid or heat inducible, and the major macrophage induced protein was not observed under any in vitro stress condition. In order to identify the proteins induced following phagocytosis two approaches are underway; (i) proteins recovered from gels are being used to raise antibody to screen a recombinant expression library, and (ii) a library of *B. abortus* transposon mutants is being screened in macrophages to identify avirulent derivatives which will be examined for expression of the appropriate proteins.

B 407 MODUS OPERANDI OF *VIBRIO CHOLERAE*: SWIM TO ARRIVE; STOP TO KILL.
THE RELATIONSHIP AMONG CHEMOTAXIS, MOTILITY AND VIRULENCE, Claudette L. Gardel and John J. Mekalanos, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

Vibrio cholerae, the causative agent of Asiatic cholera, is a highly motile, Gram negative bacterium. Many virulence factors have been identified that are important for its pathogenesis including toxins, hemagglutinins, hemolysins, and toxin coregulated pili (TCP). In order to determine the effects of motility on the expression of virulence factors, motility and chemotaxis mutants were isolated. Both spontaneous and transposon-induced mutants with altered swimming patterns were obtained. Spontaneous nonmotile and hypermotile mutants, and insertion induced chemotaxis and nonmotile mutants were examined for cholera toxin production, TCP expression, hemagglutination, hemolysis, adherence to HEp-2 cells, and ability to colonize infant mice.

In general, hypermotile mutants were defective in toxin and TCP production while nonmotile mutants showed increased toxin and TCP expression. These results suggest that virulence and motility are oppositely regulated. Although nonmotile mutants make TCP, they are unable to bind HEp-2 cells. Addition of agents that inhibit motility of wild-type bacteria abolishes adherence to eukaryotic cells, suggesting that motility itself is required for binding. Interestingly, mutants defective in chemotaxis show a striking increase in HEp-2 cell binding. *In vivo* competitions between the mutants and wild-type *V. cholerae* in the infant mouse model reveal that all three classes of mutants are dramatically attenuated. These colonization defects exhibited by the hypermotile, nonmotile and chemotaxis mutants may result from the absence of toxin and TCP, the lack of adherence and motility, or the inability to reach the intestinal epithelium.

The insertion points of the transposons are being sequenced to facilitate the identification of these putative motility and chemotaxis genes. A different method used to identify motility genes was to complement swimming mutants of *Escherichia coli* and *Salmonella typhimurium* with cloned *V. cholerae* DNA. These clones are currently being investigated. It is hoped that a better understanding of the mechanisms of motility in *V. cholerae* will yield insights into pathogenesis.

B 408 METHODS FOR THE IDENTIFICATION OF VIRULENCE GENES EXPRESSED IN *MYCOBACTERIUM TUBERCULOSIS* STRAIN H37RV, Lynne C. Kikuta-Oshima, C. Harold King, Thomas M. Shinnick, and Frederick D. Quinn, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333.

Dramatic increases in infections due to *Mycobacterium tuberculosis* over the past few years have prompted renewed efforts to characterize its virulence factors and other aspects of the pathogenesis of this disease. Attachment, invasion, and intracellular growth within macrophages may be some of the most important components in human tuberculosis pathogenesis. Our goal was to understand aspects of macrophage-bacterial interactions and identify *M. tuberculosis* genes expressed specifically during macrophage association. To identify bacterial genes, expressed under a variety of conditions we have developed a RNA subtractive hybridization method (RSH). The efficiency and usefulness of this method was initially examined by identifying genes that are expressed in the virulent H37Rv strain of *M. tuberculosis* that are not expressed in the less virulent strain, H37Ra. cDNA was made to the extracted RNA from strain H37Rv and hybridized to an excess of RNA from strain H37Ra. The subtraction products were made double stranded, PCR-amplified and used as Southern blot probes. Approximately 8.8 kilobases (kb) of DNA were identified on Southern blots. These genes, constitutively expressed by H37Rv, have been identified from a H37Rv *PstI* library. Restriction endonulcease analysis and sequence analysis are being performed on the clones from the H37Rv *PstI* library. The H37Rv strain has been shown to attach and invade J774 mouse macrophage cell line. We are in the process of identifying the bacterial genes and gene products involved in these aspects of disease by isolating bacterial RNA from various time-points after the addition of bacteria to J774 macrophage cultures. Once identified, these bacterial genes and gene products will give us a novel perspective on this disease.

B 409 IDENTIFICATION OF A PHASE VARIABLE EPITOPE IN THE LIPOOLIGOSACCHARIDE OF HAEMOPHILUS SOMNUS DISEASE ISOLATES WITH MONOCLONAL ANTIBODIES, Thomas J. Inzana, Anthony A. Campagnari, Alan J. Lesse, and Michael A. Apicella. Virginia Polytechnic Inst. State Univ., Blacksburg, VA 24061, SUNY Buffalo School of Medicine, Buffalo, NY 14215

Haemophilus somnus is a commensal of the urogenital tract and upper respiratory tract of bovines. For reasons that are not clear, the bacteria may disseminate and cause thromboembolic-meningoencephalitis, pneumonia, arthritis, and abortion. The lipooligosaccharide (LOS) of disease isolates of H. somnus undergoes antigenic phenotypic phase variation in vivo and in vitro, whereas preputial isolates do not (T. J. Inzana, R. P. Gogolewski, and L. B. Corbeil. Infect. Immun. 60:2943-2951, 1992). Monoclonal antibodies (MAb) to the LOS of H. somnus, H. influenzae H. influenzae biogroup aegyptius, and Neisseria gonorrhoeae were examined for reactivity with the LOS of H. somnus strain 738 and LOSs from 13 phase variants of strain 738. Bands of M_r 4200 to 4300 reacted with 5 of 6 MAb to H. somnus LOS, 1 MAb to N. gonorrhoeae LOS, 3 MAb to H. influenzae biogroup aegyptius, and 1 MAb to H. influenzae LOS. H. somnus LOS bands < 4000 M_r did not react with any MAb. All phase variants of strain 738 tested reacted with the same MAb as strain 738, but only if LOS bands with a M_r > 4000 were produced. Thus, at least 1 phase variable, antigenic epitope seems to be conserved in some phase variants of H. somnus. Colony blots confirmed that strain 738 underwent phase variation, based on reactivity with MAbs, at a high rate (>20%). H. somnus preputial isolate 1P produced a single LOS band of M_r 3300, did not react with any of the MAb reactive with strain 738, and did not undergo LOS phase variation after 19 clonal passages. Strain 1P may be useful as a genetic recipient to identify the phase variable LOS genes of virulent isolates.

B 410 MUTATIONS IN PEPTIDE PERMEASES FROM *STREPTOCOCCUS PNEUMONIAE* MODULATE THE PROCESS OF NATURAL TRANSFORMATION, H. Robert Masure, Ann Naughton and Barbara J. Pearce, Laboratory of Molecular Infectious Diseases, The Rockefeller University, New York, NY 10021

There has been a world wide emergence of penicillin resistant strains of *S. pneumoniae*. Recent data suggests that these strains arose by transformation (Tfn) mediated horizontal gene transfer. The process of natural Tfn occurs in a cell density dependent manner during a competent state which is triggered by unknown signalling mechanisms. To identify regulatory elements that control this process, we screened a bank of mutants with defects in exported proteins for a decrease in Tfn efficiency. Genetic analysis of a mutant that met this criteria revealed a locus (*plpA*: **p**ermease **l**ike **p**rotein) that encodes a member of the family of protein dependent peptide permeases responsible for the binding and transport of small molecular weight extracellular oligopeptides. The derived nucleotide sequence of PlpA was 60% identical and 80% similar to AmiA a previously identified permease from pneumococcus. Both PlpA and AmiA are similar to SpoOKA from *Bacillus subtillis* which controls competence in this organism. Genetic analysis of the region downstream from *plpA* did not reveal transport related genes characteristic of most peptide permeases. This suggests the promiscous use of other permease elements for substrate transport. Compared to the parental strain, mutants with defects in *plpA* showed a 10 fold decrease in Tfn efficiency, while mutants with defects in the *ami* locus were 1.4 to 4 fold more efficient. Since, these mutants would fail to deliver their peptide substrates into the bacteria, we propose that these peptides serve as cell signalling molecules that modulate the cell density dependent induction of the competent state and that the peptide permeases act not only to reclaim extracellular peptides for metabolic recycling but also mediate the transport of these cellular messengers.

B 411 GENETIC ANALYSIS OF VIRULENCE REGULATION IN *BORDETELLA PERTUSSIS*. Tod Merkel and Scott Stibitz* NIDR/NIH, 9000 Rockville Pike, Bethesda MD 20892, and *CBER/FDA, 8800 Rockville Pike Bethesda MD 20892.

A search for mutations defining additional regulators in the putative *vir* controlled regulatory cascade was undertaken. A derivative of *Bordetella pertussis* Tohama I carrying the gene fusions *ptx:phoA* and *fhaB:lacZ* was subjected to chemical mutagenesis. Pho$^-$ and non-hemolytic colonies were chosen for further analysis. The phenotype of these mutants was further characterized by quantitative determinations of *ptx* and *fha* transcription using the reporter genes described above. Mutants which had reduced or no *ptx:phoA* transcription, and were non-hemolytic, but still maintained *fhaB:lacZ* transcriptional activity were identified. All of the mutations were mapped using a conjugative mapping system recently developed in our laboratory and fell into several discrete classes.

B 412 THE TOXR PROTEIN OF *VIBRIO CHOLERAE*: ANALYSIS OF THE FUNCTION OF THE PERIPLASMIC DOMAIN *IN VITRO* AND *IN VIVO*, Karen M. Ottemann and John J. Mekalanos, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

The ToxR protein is a transcriptional activator of several virulence factors of *V. cholerae*, including cholera toxin, the toxin coregulated pilus, the accessory colonization factor, and at least 17 other proteins. The ToxR protein is a transmembrane protein that has its amino-terminal two thirds in the cytoplasm, and its carboxy-terminal one third in the periplasm. Previous analysis of ToxR structure using alkaline phosphatase fusion proteins [Miller *et al.*, Cell 48:271] suggested that ToxR activated transcription as a dimer. In order to test this, a series of fusion proteins has been constructed between the cytoplasmic portion of ToxR and several dimeric and monomeric proteins to replace the periplasmic and/or transmembrane regions. ToxR fused at amino acid 211 (just past the transmembrane region) to either a dimerized protein (alkaline phosphatase, ToxR-PhoA), a dimerization domain (GCN4 leucine zipper, ToxR-GCN4-M) or a monomeric protein (b-lactamase, ToxR-Bla) were able to activate transcription of the ToxR-regulon to 100%, 25% and 100%, respectively, of wild type levels. The ToxR-GCN4-M protein produces breakdown products by western blot analysis, indicating it may be less stable than the other two fusion proteins. In addition, *V. cholerae* strains containing the ToxR-GCN4-M and ToxR-Bla fusion proteins were seven-eight fold reduced in ability to colonize the intestine of suckling mice. ToxR fused to the leucine zipper of GCN4 at amino acid 182 (creating a cytoplasmic protein, ToxR-GCN4-C) was unable to activate transcription of the ToxR regulon and was severely impaired in the mouse colonization model. In addition, when the transmembrane region of the ToxR-PhoA was replaced with a heterologous transmembrane region, this decreased the activity of the fusion protein two-fold. These experiments have lead us to conclude that the ToxR protein is surprisingly tolerant of substitutions of its various domains, but must be inserted in the membrane. Replacement of the periplasmic portion of ToxR has little effect on its ability to activate transcription of the ToxR-regulon *in vitro*, but a more dramatic effect on ability of *V. cholerae* to colonize suckling mice. This may reflect a greater need for correct ToxR structure and function *in vivo*.

B 413 IDENTIFICATION OF *Salmonella* GENES REQUIRED FOR INDUCTION OF FILAMENTOUS LYSOSOMES, Murry A. Stein, Ka Yin Leung, Michael B. Zwick, Francisco Garcia del-Portillo, and B. Brett Finlay. Biotechnology Laboratory, University of British Columbia, Room 237-6174 University Boulevard, Vancouver, B. C. Canada V6T 1Z3

Salmonella species invade and replicate within epithelial cells residing in membrane bound vacuoles during the entire intracellular phase. In HeLa epithelial cells intracellular *Salmonella* induce the formation of stable filamentous structures containing lysosomal membrane glycoproteins (F. Garcia-del Portillo *et al.* 1993. Proc. Natl. Acad. Sci. U.S.A. *In Press.*). We named these structures *Salmonella*- induced filaments (Sif). They appear 4-6 hours after *Salmonella* invasion and are not found in uninfected cells or cells infected with other invasive pathogens (e.g., *Yersinia pseudotuberculosis*). The formation of these filaments requires viable intracellular bacteria and is blocked by vacuolar acidification inhibitors suggesting that inducible bacterial products mediate Sif formation. A *S. typhimurium* mutant, obtained by transposon mutagenesis with Tn10dCm, cannot induce filament formation (*sif*$^-$). Sequence analysis of the *sif*-associated genes linked to the transposon revealed that the transposon resides within a genetic region with high degree of similarity to the *Escherichia coli* spermidine and putrescine transport system genes. This polyamine transport system maps at 15 minutes on the *E. coli* chromosome and contains the genes *pot A,B,C,* and *D*. Using probes derived from the transposon mutated gene, the wild type *S. typhimurium sif*-associated gene was cloned. The cloned *sif*-associated gene restored filament production when transformed into the Sif$^-$ transposon mutant. Sequence analysis of the cloned wild type genes has identified, in addition to those genes with similarity to the *E. coli* polyamine uptake system genes, an open reading frame without similarity to previously described genes. Studies are ongoing to determine the mechanism of Sif formation and their role in pathogenesis.

B 414 GENETIC MAPPING BY CONJUGATION IN *BORDETELLA PERTUSSIS*: IDENTIFICATION OF A NEW LOCUS AFFECTING TOXIN EXPRESSION. Scott Stibitz and Nicholas Carbonetti*, Division of Bacterial Products, CBER/FDA, 8800 Rockville Pike, Bethesda, MD 20895, and *Dept. of Microbiology and Immunology, UMAB School of Medicine, Baltimore MD 21201.

Hfr donor strains of *Bordetella pertussis* were created by integration of a plasmid vector containing *oriT* and the *tra* genes of the broad host range plasmid RK2 at various sites around the chromosome of *B. pertussis* Tohama I. These strains were used as genetic donors in crosses with *B. pertussis* recipients having a mutant phenotype. Loci governing mutant phenotypes were mapped by examining genetic linkage to a set of selectable markers introduced at various positions in the chromosome. Trial mapping experiments were performed using *ptx*:*phoA* and *fhaB*:*lacZ* alleles for which a physical location has previously been determined, as well as mutations leading to streptomycin, nalidixic acid, rifampicin, and kasugamycin resistance. For *ptx* and *fhaB* the position determined by genetic mapping was consistent with their location on the physical map of Tohama I. The system was utilized to map two mutations which gave a phenotype consistent with mutations affecting a putative intermediate regulator in a *vir*-controlled regulatory cascade. These mutations mapped at or near the same location. Three factor crosses were performed to determine the linear order of markers in this vicinity.

B 415 THE CYSTEINE PROTEASE OF *GIARDIA LAMBLIA*: STRUCTURE, LOCALIZATION, AND FUNCTIONAL ANALYSIS, Wendy L. Ward and James H. McKerrow, Department of Pathology, VAMC, University of California, San Francisco, CA 94121

Proteolytic enzymes are known to be key enzymes in the pathogenesis of many microbes. We are therefore investigating the cysteine protease of G. lamblia, focusing on sequence, structure, subcellular location, and function. A fragment of the gene was amplified by PCR using degenerate primers designed around conserved motifs flanking the active sites of other parasite proteases. This fragment was used as a probe to locate the intact gene in a genomic library. The gene sequence shows marked homology to other parasite proteases, and displays features characteristic to giardia, including absence of inrons, a S.D.-like box, and a stop codon shortly followed by AGTPuAAPyr. Western blot of sonicated trophozoites, performed under reducing conditions and probed for cysteine proteases identifies a single, sharp 29 kd band, corresponding to the deduced size of the cloned gene. Studies of the structure, function, and molecular evolution of the cysteine protease are now underway. Using Z-F-R-MNA, a specific fluorescent substrate, we localized the cysteine protease to peripheral vacuoles, which release their contents during excystation of giardia. We have also shown that synthetic fluoromethylketones inhibit this protease in both cytoplasmic extracts and in cultures. The inhibitors do not significantly interfere with replication or encystation, suggesting that the primary role of the cysteine protease is in excystation.

Export and Cell Surface Localization of Virulence Factors

B 500 A SECRETED LECTIN FROM *Aeromonas hydrophila* WITH AFFINITY FOR COLLAGEN, Felipe Ascencio[1], Timothy R. Hirst[2], and Torkel Wadstrom[3]. [1]Department of Marine Pathology, Center for Biological Research, La Paz BCS 23,000 Mexico; [2]The Biological Laboratory, University of Kent at Canterbury, Kent CT2 7NJ UK; and [3]Department of Medical Microbiology, University of Lund, S-222 34 Lund Sweden.

The gastrointestinal pathogen *A. hydrophila* produces an extracellulary and cell-associate lectin with affinity for the fiber-forming collagen type I (CNBP). The extracellular CNBP presented in the culture fluid supernatant was purified by sequential ammonium sulfate precipitation, sieve chromatography, and affinity chromatography. The purified CNBP was homogeneous in SDS-PAGE, and has a M_r of ca. 98 kDa. CNBr-cleavage of the CNBP destroyed the collagen-binding activity; however, enzimatic digestion with *Staphylococcus aureus* V8 protease generated a polypeptide (M_r of ca. 30 kDa) containing the collagen-binding domain. Binding of collagen by the CNBP is restricted to the -1(I) chain of collagen type I, and seems to involve both the carbohydrate moieties and certain peptide sequences of collagen. The CNBP is secreted to the growth medium throughout all stages of cell growth and under a variety of culture conditions. The process governing CNBP secretion appears to be different from the pleiotrophic secretory system responsable for releasing aerolysin to the culture medium.

B 501 THE *DE NOVO* SYNTHESIS AND MUTAGENESIS OF A GENE ENCODING THE CATALYTIC DOMAIN OF DIPHTHERIA TOXIN, Steven R. Blanke, Kathy Huang, Brenda A. Wilson, and R. John Collier, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02158

Diphtheria toxin is the major virulence factor secreted by *Corynebacterium diphtheriae*. Subsequent to receptor-mediated endocytosis and proteolytic processing, the N-terminal domain (DTA) catalyzes the ADP-ribosylation of eukaryotic elongation factor 2 (EF-2), resulting in cessation of protein synthesis and ensuing cell death. A persistent problem in studying the mechanism of intoxication at the molecular level has been the inability to procure sufficient quantities of mutant toxins for detailed structural, biochemical, and biophysical investigations. Pursuant to delineating the fine structural details that dictate toxin function, including receptor binding, membrane translocation into the cytosol, and the mechanism of cell death, we have designed and chemically synthesized a gene encoding DTA. The gene design included optimal codon usage to reflect the bias discovered for highly expressed proteins in *E. coli*. Furthermore, this approach allowed us to incorporate a plethora of unique restriction sites, facilitating further genetic engineering and mutagenesis. We have measured high levels of DTA expression in *E. coli* using the synthetic gene under control of both the tac and T-7 promoters. A polyhistidine fusion peptide engineered onto the N-terminus of DTA, has allowed for the development of a rapid, single-step purification under non-denaturing conditions utilizing nickel chelate affinity chromatography. The polyhistidine fusion peptide can be readily cleaved at an engineered thrombin site, yielding authentic DTA. The overexpressed recombinant protein exhibits essentially identical properties to those of DTA obtained from *C. diphtheriae*. An extensive library of mutant toxins has been generated. In particular, a number of residues have been characterized which are critical for recognition and binding of the substrate in the ADP-ribosylation reaction. This represents the first example of *de novo* synthesis of a gene encoding a bacterial virulence factor, and has provided us with a valuable tool for studying the role of the toxin in *C. diphtheriae* pathogenesis at the molecular level.

B 502 IDENTIFICATION AND LOCALIZATION OF ACCESSORY PROTEINS NECESSARY FOR EXPORT OF PERTUSSIS TOXIN FROM *BORDETELLA PERTUSSIS*, Drusilla L. Burns, and Frederick D. Johnson, Division of Bacterial Products, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

Pertussis toxin plays a role in the pathogenesis of *Bordetella pertussis*. Since *B. pertussis* mutants which are defective in secretion of pertussis toxin are less virulent than the parent strain [Weiss and Goodwin (1989) *Infect. Immun.* 57: 3757], secretion of pertussis toxin, not simply production of the toxin, appears to be important for pathogenesis.

Previously, a region of the chromosome important for secretion of this toxin was identified [Weiss *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90: 2970]. This region, termed *ptl* for pertussis-toxin-liberation, contains eight open reading frames (orfs), each of which would encode a protein homologous to one of the VirB proteins produced by *Agrobacterium tumefaciens* which are believed to be involved in transport of T-DNA across bacterial membranes [Zambryski (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:465]. In this study, we identified three of the Ptl proteins and have determined their localization within the bacterial cell.

Ptl proteins were identified by amplifying each orf using PCR, expressing recombinant forms of the proteins in *Escherichia coli*, isolating these proteins, immunizing mice with the preparations, and using the resulting sera to probe extracts of *B. pertussis* for the Ptl proteins. Proteins recognized by PtlE, PtlF, and PtlG antisera were found in extracts of virulent *B. pertussis* but not in the avirulent form of the organism. All three of the Ptl proteins were localized to the membrane fraction of *B. pertussis*.

The membrane localization of the Ptl proteins and analogies with the VirB protein system suggest that Ptl proteins may form a complex in the membrane which facilitates transport of pertussis toxin across membrane barriers.

B 503 **TOPOLOGY OF *MYCOPLASMA* VARIANT SURFACE LIPOPROTEINS,** Catherine M. Cleavinger and Kim S. Wise, Dept. of Molecular Microbiology and Immunology, University of Missouri-Columbia, Columbia, MO 65212

Pathogens in the genus *Mycoplasma* possess a single membrane and lack cell walls, which places all environmentally interactive components on this unique prokaryotic surface structure. Highly variant lipoproteins (Vlps) have been identified as major surface components of *Mycoplasmas*, and are likely involved in transport, signal transduction and general mechanisms for host adaptation, including avoidance and modulation of the immune system.

To evaluate the roles of Vlps at the mycoplasma surface, the processing and topology of VlpC, a prototype variant lipoprotein of *M. hyorhinis*, was examined. Protein sequences deduced from several *vlp* genes, indicate a conserved bacterial lipoprotein signal sequence. To establish this processing pathway, the predicted amino acid sequence of VlpC was exploited, in which 5 Leu residues are all contained solely within the signal sequence. Selective inability to metabolically label VlpC with [^{14}C]Leucine indicated efficient processing of the VlpC lipoprotein signal in mycoplasma, supporting VlpC anchorage in the membrane by the N-terminal, acylated Cys residue. The lack of a membrane spanning segment argues against a role for Vlps in signal transduction and supports a primary role in variation of surface structural mosaics.

VlpC topology and surface-exposed epitopes were mapped using a panel of MAbs. Two external regions (region II, containing discrete blocks of similar amino acid sequences interspersed with unique regions; and region III, composed of distinctive C-terminal repetitive structures) comprise the mature Vlp structure. Surface-binding MAbs (*i*) specific for VlpC, (*ii*) mediating C'-dependent cytolysis, and (*iii*) directed to an epitope shared by Vlps were mapped to region II by *ΔTnphoA* mutagenesis of recombinant *vlpC* coding region in *E. coli*. Active *vlpC::phoA* fusions expressing 54 kDa and 57 kDa fusion proteins, were examined by Western blot analysis to establish the presence of VlpC epitopes. This defined region II as a surface accessible domain, and implicated variable Vlp sequences in this region as potential sources of topological diversification in the Vlp system, subject to selective pressure by the host immune response.

B 504 THE YopB PROTEIN OF *YERSINIA PSEUDOTUBERCULOSIS* IS INVOLVED IN THE TRANSLOCATION OF THE YopE CYTOTOXIN ACROSS THE TARGET CELL MEMBRANE, Sebastian Håkansson, Roland Rosqvist and Hans Wolf-Watz, Department of Cell and Molecular Biology, University of Umeå, 901 87 Umeå, Sweden.

Pathogenic species of *Yersinia* all have the capability of resisting phagocytosis by professional macrophages. This process is dependent on the expression of the YopE protein, which induces disruption of the host cell actin microfilaments. The action of the YopE cytotoxin requires that the protein is actively translocated across the target cell membrane in a polarized manner. An *in frame* deletion in the *yopB* gene, located on the virulence plasmid of *Y. pseudotuberculosis*, rendered the corresponding mutant strain unable to translocate the YopE cytotoxin through the plasma membrane. Using immunofluorescence techniques on HeLa cells infected with the specific YopB mutant, it was observed that YopE was deposited on localized spots on about 10-20% of the bacteria that were associated with HeLa cells. No localized staining of YopE was observed when the wild-type strain was analyzed. This observation indicates that the *yopB* mutant strain can not translocate the YopE protein. The *yopB* mutant strain was incapacitated in its ability to inhibit phagocytosis and was no longer virulent for orally challenged BALB/c mice.

B 505 THE PSEUDOMONAS SYRINGAE PV. SYRINGAE 61 hrp CLUSTER MEDIATES EARLY EVENTS IN THE SECRETION OF YERSINIA ENTEROCOLITICA YopE, Steven W. Hutcheson and Michael C. Lidell, Department of Botany, University of Maryland, College Park, MD 20742

The Pseudomonas syringae pv. syringae 61 (Pss61) hrp cluster contains at least 17 known genes organized into 7 transcriptional units which are essential for phytopathogenicity. The cluster appears to encode an autonomous system for the Sec-independent secretion of a glycine-rich, heat stable 34.7 kDa protein which controls the host range and pathogenicity of the host bacterium. The entire cluster has been cloned as a 31 kb fragment and shown to be functional in Escherichia coli strains. Sequence analysis has revealed Hrp homologues to the Yersinia YscC (HrpH), YscJ (HrpY) and LcrD (HrpI) families of proteins and to Salmonella FliG (HrpK), the FliI family (HrpO) and FliJ (HrpP) proteins. In Yersinia strains, YcsC, YscJ and LcrD are associated with Yops protein secretion and exhibit high homology to the Hrp equivalents. To determine if the Pss61 hrp cluster forms a general pathway for protein secretion, E. coli strains carrying the plasmid-borne Pss61 hrp cluster (pHIR11) were screened for their ability to secrete YopE. The plasmids, pMS3 (yopE$^+$ sycE$^+$) and pCL5 (virF; gifts of G. Cornelis) were transformed into E. coli MC4100 (pHIR11) and YopE production monitored by using immunoblots of cell fractions. A 25 kDa protein could be detected in total lysates of induced cells. Culture filtrates and osmotic shock fluids failed to exhibit evidence of hrp-dependent YopE secretion. YopE, however, was found to associate with envelope membranes only when the hrp genes are expressed. The role of specific hrp genes in mediating the apparent early steps in secretion will be reported. (This work supported by grants from NSF, USDA-NRICGP and MIANR-MAES)

B 506 ANALYSIS OF MEMBRANE-ASSOCIATED HEMOLYTIC ACTIVITIES IN MYCOPLASMAS, F. Chris Minion and K. Jarvill-Taylor, Veterinary Medical Research Institute, Iowa State University, Ames, IA 50011

The mollicutes are cell wall-less procaryotes many of which cause disease in humans and animals. Because mycoplasmas release few toxins, much effort has been directed at membrane activities that are believed to contribute to the disease process. Analysis of potential virulence factors has been difficult because of limited genetic tools. In earlier studies, an unusual membrane-associated hemolysin was detected in the rodent pathogen *Mycoplasma pulmonis*. The unusual nature of this hemolytic activity suggested a role in growth and possibly in virulence as well because it correlated with a requirement for membrane constituents which must be acquired from the host environment. Acquisition of these materials could initiate cellular lysis or other aberrant cell responses and thereby contribute to virulence. The studies reported here were undertaken to determine if membrane-associated hemolysins were common in mycoplasmas and to enhance our understanding of their function.

Approximately 25 species of mollicutes were examined for membrane-associated hemolytic activity using a mouse trypsinized red blood cell assay. Most species, except for the arginine-utilizing species and the two intracellular human pathogens, *M. penetrans* and *M. incognitus*, were hemolytic. Monoclonal antibodies (Mabs) that block hemolysin activity in *M. pulmonis* were found to inhibit hemolytic activity in all but two mycoplasma species. These Mabs also reacted by ELISA with most mycoplasma species tested.

Hemolysis inhibition studies were performed with *M. pulmonis* to identify the red cell receptor and try to elucidate its mechanism of action. In general, any treatment shown to block adherence to red cells also blocked hemolysis including trypsin treatment of mycoplasmas or inclusion of dextran sulfate or fucoidan. Hemolysis activity displayed a unique inhibition spectrum with cholesterol derivatives suggesting that cholesterol may serve as the red cell receptor. In summary, these studies showed that the mycoplasma membrane-associated hemolytic activity was unique and unlike other known hemolysins or cytolysins.

B 507 PERTURBATION OF THE STRUCTURE AND FUNCTION OF MACROPHAGES BY AN ESSENTIAL VIRULENCE PROTEIN OF *YERSINIA ENTEROCOLITICA*, Roy M. Robins-Browne and Elizabeth L. Hartland, Departments of Microbiology, Royal Children's Hospital and University of Melbourne, Parkville, Victoria 3052, Australia.

Pathogenic strains of *Y. enterocolitica* are able survive and even proliferate within macrophages and polymorphonuclear leukocytes. The resistance of these bacteria to killing by phagocytes is mediated in part by chromosomal genes and partly by genes carried on a ~70-kilobase virulence plasmid (pYV). Plasmid-borne genes appear to reduce the bactericidal capacity of phagocytes by mediating inhibition of the respiratory burst, thus interfering with the production of bactericidal oxygen species. In order to identify the gene(s) responsible for this property we used transposon Tn*2507* to mutagenize a virulent strain of *Y. enterocolitica* of serogroup O9. This strategy yielded several mutants, including three which had lost the capacity to inhibit the respiratory burst of macrophages. Although different from each other, all three mutants failed to produce YopD, a 33-kDa protein encoded by pYV. These mutants were also less able to resist phagocytosis than wild-type *Y. enterocolitica* or bacteria carrying mutations in other plasmid-borne genes. YopD$^-$ mutants were also completely avirulent when inoculated intraperitoneally into mice. Preliminary data suggest that YopD acts by disrupting cytoskeletal integrity, thus interfering with the assembly of NADPH oxidase, an essential enzyme for respiratory burst activity. These findings indicate that pYV, and more specifically YopD, play an essential role in the pathogenesis of yersiniosis by interfering with the microbicidal activity of macrophages.

B 508 SPECIFIC CHAPERONES REQUIRED FOR SECRETION OF YOP PROTEINS BY YERSINIA

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Secretion of the Yop proteins by Yersinia occurs by a mechanism unrelated to other systems and hence called type III secretion pathway. The minimal domain required for secretion is different for each Yop in terms of length and primary structure. No cleavage of the Yops occurs during export. The secretion system is made of several proteins encoded by the virA, virB and virC loci. These proteins are required for secretion of the complete panoply of Yop proteins. Most of them are predicted or have been shown to be associated with the membranes. Secretion also requires several factors that appear to be specific for individual Yops. These factors called Syc (for Specific Yop Chaperones) are small acidic proteins found in the cytoplasm. So far, we identified three Syc proteins. SycE, SycH and SycD are required for secretion of YopE, YopH and YopB/YopD respectively. The syc genes are localised close to the genes encoding the Yop protein they serve. SycE and SycH are similar to each other whereas SycD is substantially different. SycE and SycH have been shown by independant methods to bind the aminoterminal part of their target Yop. Their absence promotes the intracellular accumulation of the corresponding Yop precursors. We inferred from these data that the Syc proteins are required to stabilize and maybe to pilot the Yop proteins. They may represent the archetype of a new class of chaperone proteins. As for the yop genes, the syc genes are only expressed at 37°C. Expression of sycE and sycD requires VirF, the transcriptional activator of the *yop* regulon, whereas sycH does not.

Late Abstracts

THE LEWIS B BLOOD-GROUP ANTIGEN IS A FUNCTIONAL RECEPTOR FOR *HELICOBACTER PYLORI*, MEDIATING SPECIFIC ATTACHMENT TO SURFACE MUCOUS CELLS IN THE HUMAN GASTRIC EPITHELIUM.

Thomas Borén[1], Per Falk[2], Kevin A. Roth[2,3], Göran Larson[4], Staffan Normark[1], Departments of Molecular Microbiology[1], Molecular Biology and Pharmacology[2], Pathology,[3] Washington University School of Medicine, St. Louis, MO, 63110, Department of Clinical Chemistry[4], University of Göteborg, Sahlgren's Hospital, S-413 45 Göteborg, Sweden.

Helicobacter pylori, a human-specific pathogen highly associated with the development of gastric ulcer disease and gastric adenocarcinoma exhibits cell lineage-specific tropism in the gastric epithelium. In this report we demonstrate that the Lewis^b (Le^b) (Fucα1.2Galβ1.3 (Fucα1.4) GlcNAcβ1.3Galβ14.Glc) blood-group antigen expressed on gastric surface mucous cells is a functional receptor for *H. pylori* attachment. Human milk glycoproteins presenting the Le^b antigen inhibited bacterial binding. The fine detailed specificity of *H. pylori* for Le^b was assessed using panels of fucosylated neoglycoproteins and glycosphingolipids. Attachment of *H. pylori* to human gastric surface mucous cells *in situ* was specifically inhibited by monoclonal antibodies against the Le^b antigen. A panel of free fucosylated oligosaccharides were analyzed. The H I, Le^b and Le^y oligosaccharides reduced bacterial binding. This could be explained by a relaxation in receptor specificity for free oligosaccharides, a mechanism described for several lectins. The more than 1000-fold difference in saccharide concentration needed for inhibition using free oligosaccharides compared to neoglycoproteins emphasizes the importance of multivalency for the bacterial-receptor interaction. As a biological consequence of the high receptor specificity of *H. pylori* for Le^b, gastric tissue lacking Le^b expression failed to support bacterial binding. The Le^b antigen substituted with a blood group A determinant did not interact with *H. pylori* suggesting that the availability of *H.pylori* receptors might be reduced in individuals of blood group A and B phenotypes, as compared to individuals of blood group O phenotype. This could possibly explain previous epidemiological observations that individuals of blood group O phenotype run a greater risk for developing gastric ulcers. Consequently, anti-microbial therapy based on anti-adhesive Le^b-derived carbohydrates (soluble receptor analogues) may be a rational future therapy for *H. pylori* infections and gastric ulcer disease.

THE NORMAL INTESTINAL MICROFLORA AND ITS EFFECTS ON GLYCOSYLATION, GENE INDUCTION AND CELLULAR PROLIFERATION IN THE EPITHELIUM OF THE SMALL INTESTINE, Lynn Bry, Per Falk, Tore Midtvedt*, Jeffrey I. Gordon, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, *Department of Medical Microbial Ecology, Karolinska Institute, Stockholm, SWEDEN

We have investigated the effects of the normal gut microflora on the development and proliferation of the small intestinal epithelium in a model system using NMR1 mice. These mice have been maintained in one of four environments, germ free, ex-germ free, conventional and monocontaminated with the mucin degrading strain, *Peptostreptococcus micros.* In each case we have used a variety of mRNA, antibody and lectin probes to assess the effects of these alterations on the intestinal epithelium. Lectin probes including *Ulex europaeus* I and AAA (*Anguilla anguilla*) reveal substantial differences in the patterns of glycosylation among the germ free, ex-germ free, conventional and monocontaminated small bowels. These differences become apparent only during and after the weaning period, suggesting that factors other than changes in the microbiota, e.g. hormonal and dietary factors, may be involved as well. The changes include a loss of fucose epitopes in the the goblet cell lineage in germ free animals while conventional and ex-germ free animals acquire staining in the enterocytic lineage. Monocontaminated animals demonstrate a partial activity in the enterocytic lineage. We believe that these alterations arise from the transcriptional induction of novel glycosyltransferases. We have also noted perturbations in the levels of cellular proliferation in germ free and conventional animals as detected by the labeling of actively dividing cells with 5'-Bromo-2'-deoxyuridine. These perturbations extend throughout the GI tract but do not become significant until after the first week of life. In conclusion, we have detected a variety of changes in the intestinal epithelium induced exclusively by the presence of the normal intestinal microflora. We believe that these changes may have an effect on establishing and on maintaining the mucosal barrier functions that play a significant role in preventing colonization and invasion by pathogenic microorganisms.

MacMARCKS, a protein kinase C substrate associated with the phagocytosis of Gram negative bacteria by macrophages

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MacMARCKS, a member of the MARCKS family of PKC substrates, is restricted in its expression and is highly enriched in macrophages which have interacted with Gram negative bacteria. MacMARCKS binds calmodulin and actin in a phosphorylation regulated manner and appears to function as a molecular integrator of the calcium/calmodulin and signal transduction pathways in the regulation of actin-membrane interactions. Upon phagocytosis of bacteria, MacMARCKS associates tightly with phagosomes after they have been denuded of actin, and becomes colocalized with the lysosome marker, LAMP-1, upon phagosome-lysosome fusion. The role of MacMARCKS in regulating membrane flow to the phagosome and the effect of virulence factors on this pathway will be discussed.

EXPRESSION OF A NOVEL CONTACT-HEMOLYTIC ACTIVITY BY *E. COLI*, Bernt Eric Uhlin and Yoshimitsu Mizunoe, Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden.

Hemolysin/cytotoxin production has been shown to be associated with virulence of a number of bacterial species and specific chromosomal or plasmid-borne genetic determinants are known in pathogenic isolates. Here we report that normally non-pathogenic *E. coli* strains also may exhibit such hemolytic/cytotoxic activity. While studying how mutations in genes for global regulators might affect expression of several *E. coli* genes we found that a novel, hitherto cryptic, hemolysin/cytotoxin gene could be derepressed. The bacteria showed cytotoxic activity towards HeLa cells *in vitro* and an apparent contact-dependent lytic activity towards erythrocytes. Genetic analysis and molecular cloning revealed that a gene mediating the cytotoxic activity resided within a 1.6-kb DNA fragment in the *E. coli* chromosome. The primary structure of the protein product, as predicted from the nucleotide sequence, did not show any homology to those of other, previously characterized, hemolysins or cytotoxins. Homologous DNA sequences were found in several different isolates of *E. coli* and the gene seemed strictly regulated in wild type bacterial strains.