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ABSTRACTS

Genomics of microbial pathogens and host–pathogen interactions

166: Genomic plasticity of *Helicobacter pylori* and impact on evolution of novel virulence mechanisms

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Although *H. pylori* has been connected to gastric cancer, it has never been seen as a big threat to human health in the South Asian countries and particularly in India. This prompted us to look into the population structure of *H. pylori* in India. We found that it shares genetic origins as well as its trajectory of virulence genes with the western strains. A series of putative virulence factors which are also abundant in Indian isolates, were characterized by our group. These virulence factors are encoded by the genomic plasticity region genes which constitute a putative type IV secretion system believed to be acquired horizontally. Two of these virulence factors (JH940 and HP986) potentially interacted with the human immune system under in vitro conditions. The HP986 appears to be involved in *Helicobacter* persistence. Most persistent microbes seemingly evolve strategies to foil host responses and gain a niche. However, it seems that there is fine tuning between microbial immune evasion and maintenance of the growth fitness. For example, *H. pylori* both downregulates T-cell responses through the VacA mediated cell cycle arrest, and upregulates mucosal proinflammatory pathways by CagA. Surprisingly in our studies, HP986 appears to be able to perform both the immune stimulatory and immune evasion tasks single handedly.

167: Molecular characterization of *Mycobacterium tuberculosis* PII protein

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Tuberculosis is a major killer disease worldwide. In efforts to identify new drug targets, it is important to characterize proteins of biochemical pathways critical for survival. The Nitrogen assimilation and regulation is one such step. The signaling protein PII senses intracellular carbon and nitrogen status and regulates Glutamine synthetase activity. PII protein from *M. tuberculosis* (Mtb) was cloned and expressed in *E. coli*. Peptide mass fingerprinting confirmed the purified protein as Mtb PII. Unlike *E. coli* PII, the Mtb PII did not display post-translational modification in nitrogen optimum, excess and starvation conditions indicating the inability of orthologous enzyme of *E. coli* to modify Mtb PII or a novel intrinsic character of Mtb PII. These results are strikingly new considering that PII is a universally conserved protein. Phylogenetic analysis revealed that Mtb PII and other mycobacterial PII proteins form a distinct lineage distant from the proteobacterial PII lineage. Multiple alignment using ClustalW showed that residues forming the T-loop including Tyr51 (post-translational modification site) and the ATP binding site are conserved, whereas the differences are scattered throughout the protein sequence. In order to gain structural and functional insights into Mtb PII, a homology model was generated using SWISS-MODEL, Insight II using X-ray crystal structures of orthologs 1QY7 (*Synechococcus* sp. PCC 7942) and 2PII (*E. coli*).The structure revealed that it may bind to ATP. Also, 2-ketoglutarate (intracellular carbon status indicator) binding site was predicted using *M. jannaschii* PII crystal structure (2J9E) as template. Glutaraldehyde Crosslinking study revealed that Mtb PII exists in homotrimeric state which is consistent with other PII proteins. CD spectroscopy confirmed the presence of ordered secondary structure in Mtb PII. Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC) studies confirm the binding of ATP (Kd = 6.44 ± 1.35 μM). Interaction of Mtb PII with ATP remains unaffected in the presence of 2-ketoglutarate (Kd = 8.87 ± 2.82 μM). These findings appear to be supported by the modeling studies. Mtb PII also binds to GTP (Kd = 8.09 ± 5.04 μM) and CTP (Kd = 8.44 ± 2.21 μM) with almost equal affinity as ATP. UTP (Kd = 31.5 ± 0.72 μM) and ADP (Kd = 29.3 ± 7.82 μM) show lower affinity. This had so far remained unexplored. Interaction energetics revealed the exothermic binding nature of the nucleotides to PII.

168: Unusual codon usage bias in lowly expressed genes of *Vibrio cholerae*

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The synonymous codon usage bias has been shown to be positively correlated with the potential gene expression level. However, in the present study of *Vibrio cholerae* genome, we have identified a group of 138 genes having unusually high codon usage bias despite being lowly expressed. Further analysis reveals that gene length has strong influence over the usage pattern of non-optimal codon in lowly expressed genes; the frequencies of non-optimal codons are greater in shorter genes compared to longer genes. The present study suggests that codon usage in lowly expressed genes might also be selected on to preferably use inefficiently translated codons (non-optimal codons), so as to maintain a low cellular concentration of the proteins that they encode. This would predict that low-expressed genes are also biased in codon usage, but in a way that is opposite to the bias of highly-expressed genes.

169: Genetic variations in TNF-LTA locus and susceptibility to HPV mediated cervical cancer in North Indian women

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Aim: To investigate the potential association of SNPs in TNF-LTA locus with susceptibility to HPV associated cervical cancer/precancer in North Indian women. Methods: 200 cases (150 invasive cervical cancer and 50 precancer) and 200 healthy controls were included for the present study. Checked for the presence of HPV DNA sequence by PCR. PCR–RFLP methodology was employed to identify SNPs at −238 G/A, −308 G/A, −857 C/T, −863 C/A and −1,031 T/C loci in the promoter region of TNF and at +252 A/G locus in the intron I region of the LTA. Validated by direct DNA sequencing. TNF A and LTA levels were quantitated by ELISA. Results: 82.0% of cases and 2.5% of healthy controls showed positivity for HPV DNA sequence. Out of HPV positive cases, 98.17% were infected with HPV 16 and 1.8% by HPV 18. But all HPV positive healthy controls were infected with HPV 16. Significant association was established for TNF-308 G/A (Pc 0.01) and LTA +252 A/G (Pc 0.02) polymorphisms with respect to cancer vs. controls. Similar results were obtained for both TNF −308 G/A (Pc 0.01) and LTA +252 A/G (Pc 0.024) for HPV positive cases versus controls. TNF −1,031 T/C was also significantly associated with HPV positive cases versus HPV negative cases (Pc 0.01). On the other hand, TNF-857 C/T was found to have protective effect in relation to cervical cancer (Pc 0.04). No relation was found either with HPV infection or cervical cancer for −238 G/A and −863 C/A.AGGCCT haplotype showed a protective association with cervical cancer (Pc 0.02) and HPV infection (Pc 0.01) while both AGGCCC and GGACCT haplotypes emerged as important 'risk haplotypes' for cervical cancer (Pc 0.01). Similarly, AGGCCC haplotype showed positive association for HPV infection ($Pc < 0.002$); however, GGACCT haplotype revealed significant association (Pc 0.01) with HPV negative cases than controls. Plasma TNF levels were significantly elevated in cases in compare to controls (precancer P 0.045, cancer P 0.001). LTA level was also found to be positively associated with precancer (P 0.001) in compare to control. Similar results were also obtained with respect to HPV infection status. Association of genotype with level revealed a functional correlation between TNF-308 G/A polymorphism and elevated plasma TNF levels in cases ($P = 0.03$ for GG vs. GA/AA). Conclusion: SNPs in TNF-LTA locus may act as potential biomarker for HPV infection and cervical carcinogenesis in Indian women.

170: Use of D-Limonene as an antimicrobial agent against *Hemolytic streptococci*—An in silico and in vitro approach

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Hemolytic streptococci are gram-positive, facultatively anaerobic cocci capable of hemolyzing erythrocytes. Rupture of erythrocytes and the release of hemoglobin into the surrounding fluid is called hemolysis. Penicillin is used for the treatment of hemolysis but the mechanism of penicillin activity on hemolytic streptococci is not properly understood. Studies have revealed that D-limonene acts on Ammonia Mono Oxygenase enzyme (AMO), but the hemolytic streptococci does not contain the above mentioned enzyme as it is present only in the nitrifying bacteria. Thus the studies involved, determining those protein sequences in hemolytic streptococci, similar to that of ammonia mono oxygenase using sequence analysis tools. The structure of the protein was extracted from Protein Database and the docking study of this protein with D-limonene was conducted. The interaction between D-Limonene and the protein obtained from the streptococci by using in silico approach proves that D-Limonene has the ability to inhibit the Hemolytic streptococci. Using in vitro studies D-Limonene was tested on hemolytic streptococci. The zone of inhibition was calculated after incubation period of 24 h at 370°C and compared with standard antibiotics. D-Limonene has high anti-microbial property having the zone of inhibition ranging from 1.8 to 4.9 cm for a number of microbial strains (Anti-microbial property of a compound is characterized by a zone of inhibition greater than 1.5 cm). So by in silico and in vitro analysis we can hypothesize that D-Limonene is a potent anti-microbial agent against Hemolytic streptococci.

171: Global gene expression analysis of *P. vivax* parasites causing severe malaria and interaction with the host genome

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Plasmodium vivax long thought to be a benign parasite has recently been shown to cause severe manifestations (1, 2) which include cerebral malaria, hepatic failure, renal failure etc which are associated with sequestration linked phenomena in *Plasmodium falciparum* infections. The present study uses global expressions analysis of *P. vivax* parasites involved in severe manifestations and attempts to

correlate the data with expression analysis of the human PBMC from the same cases. Initial data shows up-regulation of 1,594 genes (PlasmoDB) using pooled severe *P. vivax* samples in comparison to non severe manifestations.

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172: Host cell contact induces expression of virulence factors in *Vibrio cholerae*

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Vibrio cholerae, a gram negative, non-invasive enteric bacterium is the causative agent of the diarrheal disease cholera. The major virulence factors of *V. cholerae* are cholera toxin (CT) and a toxin-coregulated pilus (TCP), coordinately expressed with CT that greatly enhances colonization of the intestinal epithelium by the bacterium. The expression of these virulence factors is coordinately regulated by the hierarchical expression of regulatory proteins comprising the ToxR-ToxT regulon. Paradoxically, the intestinal environment may be presumed to display parameters similar to the non-permissive conditions for induction of the virulence regulon of *V. cholerae*. In vitro, the ToxR regulon is maximally expressed in cells grown at 30°C in media of starting pH 6.6 and osmolarity equivalent to 66 mM NaCl. In the intestinal lumen, the temperature is 37°C, pH is alkaline and osmolarity is thought to be equivalent to 300 mM NaCl or higher, conditions that repress expression of ToxR activated virulence factors in vitro. Furthermore, bile a major constituent of the small intestine, represses expression of virulence factors. These observations suggest that as yet unidentified signals encountered by *V. cholerae* under intestinal conditions may overcome the repression of the ToxR regulon imposed by bile, alkaline pH and 37°C temperature. Indeed, we have demonstrated that expression of both the ctxAB gene encoding CT and the tcpA gene coding for TCP as well as the toxT gene encoding the transcriptional activator of both ctxAB and tcpA is strongly induced in *V. cholerae* associated with the intestinal epithelial cell line INT407. Neither media component nor component secreted by INT-407 into the medium was responsible for the induction. Furthermore, host cell contact can overcome the repression of virulence gene expression imposed by alkaline pH, 37°C temperature and bile.

173: An integrated proteomic and transcriptomic approach to study the effect of amphotericin B on *Aspergillus fumigatus*

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Amphotericin B (AMB) is the most widely used polyene antifungal drug for the treatment of systemic fungal infections including invasive aspergillosis. It has been our aim to understand molecular targets of AMB in *Aspergillus fumigatus* (*A. fumigatus*) by genomic and proteomic approaches. In transcriptomics analysis, a total of 295 genes were found to be differentially expressed—165 up-regulated and 130 down-regulated and included many belonging to the ergosterol pathway genes, genes for cell stress proteins, cell wall proteins, transport proteins and hypothetical proteins. Proteomic profiles of *A. fumigatus* alone or *A. fumigatus* treated with AMB showed differential expression of 85 proteins—76 up-regulated and 9 down-regulated. Fourty-eight of them could be identified with high confidence and belonged to the same above catagories. Differential expression of Rho-GDP dissociation inhibitor (Rho GDI), secretory pathway GDI, vesicle-associated membrane protein (VAMP)-associated protein, clathrin, Sec 31 (subunit of exocyst complex) and RAB GTPase Ypt51, is being reported for the first time in response to an antifungal drug and may represent specific response of *A. fumigatus* to AMB. The expression of some of these genes on exposure to AMB is being validated by real time RT-PCR. The AMB responsive genes/proteins observed to be differentially expressed in *A. fumigatus*, may be further explored for novel drug development.

174: Cloning, overexpression and purification of *Leishmania donovani* enolase

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Introduction: Parasites of the genus *Leishmania* (family-Trypanosomatidae) are the causative agents of a spectrum of diseases collectively called leishmaniases. Need for alternative treatments for these diseases is highly desirable, since conventional therapeutic agents are rather toxic with emerging resistance. Th1 immune responses play an important role in mediating protection against *Leishmania*, including roles for IFN-γ, IL-12, TNF-α and NO, whereas inhibitory effects have been reported for IL-10 and TGF-β. Effective immunization against leishmaniasis in animal models can be effected by delivery of Ag encoding DNA vectors or by administration of proteins inducing Th1 response. Through proteomics, Enolase has been identified as a Th1 stimulatory protein which warrants its further evaluation as DNA Vaccination.

Objective: Cloning, overexpression and purification of *Leishmania donovani* enolase (Ld-enolase).

Methodology: PCR amplification of the Ld-enolase was performed using the oligonucleotides: 5′-GGATCCATGCCGATCCGAAAGGT TTACGCC-3′ as forward primer and 5′-GAATTCTTACGCCCAGCC GGGGGTAGCCGTA-3′ as reverse primer containing BamHI and EcoRI sites using the *L. donovani* genomic DNA as template. The amplified fragment was purified and ligated into the linearised pTZ57R/T vector and sequenced. The recombinant enzyme was then released from the vector by digestion with BamHI and EcoRI enzymes and ligated into the eukaryotic expression vector pET28a and mammalian expression vector pCDNA3.1digested with the same enzymes. The *E. coli* rosetta cells transformed with pET28a+ enolase was induced with 1 mM IPTG final concentration for 4 h and the recombinant protein was purified using Ni-NTA column at 250 mM imidazole concentration.

Result: The nucleotide sequence of Ld-enolase, on submission to NCBI, was provided the accession number—EU723850 and was further confirmed by multiple sequence alignment using CLUSTALW. The molecular-weight of recombinant enolase on SDS–PAGE was $\sim$50 kDa.

Conclusion: Like many parasite proteins that have been used for vaccination either as recombinant protein or as DNA vaccination, the

Ld-enolase may also be further evaluated for its potential prophylactic efficacy as a vaccine candidate against visceral leishmaniasis—a fatal disease.

175: A bioinformatics pipeline to identify bacterial protein drug targets: Integration of novel gene discovery and existing annotations

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With the advent of microbial whole-genome sequencing, there has been some renewed optimism that genomic knowledge will speed the development of new antimicrobials, vaccines and diagnostics, which are urgently needed. Some success stories have been reported to date; however, these have been extensive/costly endeavors involving significant laboratory requirements. Existing computational screens for identifying potential drug targets and vaccine components are not accurate enough.

The Bioinformatics for Combating Infectious Diseases (BCID) Project at Simon Fraser University is developing improved bioinformatics methods to identify anti-infective drug targets, focusing in particular on proteins that are essential for pathogens to survive in the host or cause infection. These include proteins encoded by novel, essential genes we have identified in currently annotated intergenic regions and existing genes that appear to be pathogen-specific or virulence-related. The pipeline is being developed as a series of modules that incorporates established methods, such as PSORTb, the world's most precise predictor of bacterial cell surface and secreted proteins, as well as new methods for identifying genes predominately associated with pathogens. Our initial efforts are focused on identifying targets in two pathogens: *Pseudomonas aeruginosa*, an opportunistic pathogen that plays a key role in the mortality of cystic fibrosis patients and is noted for its intrinsic antimicrobial resistance; and *Mycobacterium tuberculosis*, the causative agent of tuberculosis which is noted for its recently emerging multiply antibiotic resistant strains. As we develop and validate our bioinformatics pipeline, we will expand it to identify potential drug targets from other priority microbial pathogens. Improved bioinformatics-based drug target screening represents a promising direction to improve the efficiency of genomics-based antimicrobial drug discovery.

176: A gain-of-function mutation in a multidrug resistant clinical isolate of *M. tuberculosis*

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The function of the mce operons in the entry of *M. tuberculosis* into non-phagocytic cells is well established. In the course of an analysis for single nucleotide variations in the genome of clinical isolates, we identified a change from G to C in the intergenic region upstream to Rv0167 at 61 position in a region of putative promoter activity predicted by in silico analysis. We experimentally determined the transcription start site (TSS) by primer extension using FAM labelled primers. Our observations indicate the presence of a transcription initiation site downstream of this mutation. We further characterized the region as a strong promoter using promoterless vector with reporter assay and by comparison with other known promoters of *M. tuberculosis*. By deletion analysis the mutation was mapped downstream of the promoter and was distinct from it. We further demonstrate that this region negatively regulates the mce operon by real time experiment as well as also a heterologous promoter when cloned downstream to it. The point mutation detected in the clinical isolate abolishes the negative regulation resulting in higher expression from the associated promoters both in the clinical isolate and in the transformed *M. smegmatis* strains in comparison with the wild type sequence from *M. tuberculosis* H37Rv. Based on these observations we conclude that the clinical isolate which is also a multidrug resistant strain of *M. tuberculosis* has a gain-of-function mutation in the binding site of a negative regulator in the mce1 operon. We detect the presence of protein(s) interacting with this cis-element from *M. tuberculosis* H37Rv by electrophoretic mobility shift assay.

177: Genomic aid in search of pathogens of tomorrow and novel drug targets

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Infectious diseases such as tuberculosis (TB) are the leading cause of a large number of human deaths. *Mycobacterium tuberculosis* is the etiological agent for TB in humans. TB kills ~4,400 people every day. The current treatment regime involves taking multiple medications over an extended period of time. Microbes have evolved effective mechanisms to evade the persistent and large scale use of antibiotics. This has proved to be a major deterrent for pharmaceutical companies to invest in R&D of antibiotics. It is largely being left to Governmental agencies and Institutes to provide innovative alternatives in order to maintain the interest of Pharmaceutical companies. Microbes evolve and evade stress induced conditions by developing short cuts and adopt alternative pathways to generate energy and also switch pathways to go in 'hibernation'. Many studies have suggested the truncated citric acid cycle, with the glyoxylate shunt as an option for survival of the pathogen and pathogenesis. We propose that the precursors to support this pathway could also be generated via poly-(Beta)-hydroxybutyrate (PHB) metabolism. Comparative genomics of enzymatic conversions that can generate glyoxylate, acetyl CoA, and other enolases useful for various fatty acid transformations and PHB was done to elucidate organisms which may switch metabolic pathways in response to antimicrobials. Such organisms may become pathogenic and turn virulent. Using *M. tuberculosis* H37Rv as a model bacterium a supportive pathway has been envisaged and integrated with glyoxylate cycle to provide a complete option to pathogen for sustainable consumption of available carbon source(s). The study proposes that the enzymes of PHB synthesis and hydrolysis are possible targets for drug design, and that this should be considered when evaluating isocitrate lyase and malate synthase as targets for the ultimate killer.

178: Studies on genetic variation among *Leishmania* major, *L. tropica* and *L. donovani* by AFLP analysis

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Introduction: The intracellular parasite *Leishmania* causes a wide spectrum of human disease, ranging from self healing cutaneous leshimaniasis (caused by *L. major* and *L. tropica*) to fatal visceral leishmaniasis (caused by *L. donovani*), *Leishmania* is a digenetic obligate intracellular protozoan parasite which inhabits two highly specific hosts, sandfly where they grow in the gut as flagellated promastigote form and non-flagellated amastigote form within mammalian macrophage.
Objective: To identify DNA markers of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) for detecting genetic variation and phylogenetic structure. Methodology: Genomic DNA was isolated from *L. major*, *L. tropica* and *L. donovani* separately. Genomic DNA digestions of all three species was done by two restriction enzymes EcoRI and MseI then EcoRI and MseI adapters (Applied Biosystems, Foster City, CA, USA) was ligated to the exposed restriction sites. Pre-selective amplification was done with EcoRI and MseI preselective primers through PCR according to applied biosystems kit instructions. After that selective amplification was done using ten combinations of fluorescence labeled EcoRI and MseI selective primers separately by PCR. Finally gel based analysis of amplified fragments was done using Perkin-Elmer/Applied Biosystems automated DNA sequencer by GeneScan software.
Result: Ten combinations of selective primers detect a total of 1,487 AFLP bands, out of which 671 (45.12%) bands were polymorphic. Analysis based on polymorphic AFLP markers revealed a high genetic variation among the genome of these species. Conclusion: Genetic variation drawn from AFLP analysis in the form of molecular marker can be used to develop the understanding of drug targets against CL and VL. The species specific markers may throw the light on their genes and mechanism involving their habitat priorities for skin and visceral organs of human.

179: DnaA Protein mediated helix opening of *Mycobacterium tuberculosis* OriC occurs at a precise location and is inhibited by a putative IciA like protein

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Mycobacterium tuberculosis (*M. tb*), the pathogen that causes tuberculosis, is capable of staying asymptomatically in a latent form, persisting for years in very low replicating state, before getting reactivated to cause active infection. It is therefore important to study the molecular genetic aspects of *M.tb* chromosome replication, specifically its initiation and regulation. While the region between dnaA and dnaN gene is capable of autonomous replication, little is known about the interaction between DnaA and oriC and their negative effectors of replication. Using KMnO4 mapping assays the sequences involved in open complex formation within oriC, mediated by *M. tb* DnaA protein, were found to reside at nucleotide position −500 and −517 with respect to the start of dnaN gene. Contrary to *E. coli*, the M.tb DnaA in presence of non-hydrolysable analogue of ATP (ATPγS) was unable to participate in helix opening pointing to the importance of ATP hydrolysis. Surprisingly, ATPase activity in the presence of supercoiled template was higher than that observed for DnaA box alone. We also show that recombinant Rv1985c, a putative IciA identified on the basis of homology to *E. coli* IciA protein, could inhibit in-vitro helix opening mediated by DnaA protein, provided the open complex formation had not initiated. It does so by specifically binding to A+T rich region of *M.tb* oriC, as seen from electrophoretic mobility shift assay. IciA could also inhibit in-vitro replication of plasmid carrying the *M. tb* origin of replication. Gel filtration analysis revealed that rIciA was a dimer and secondary structure analysis of IciA revealed almost equal α helix and β sheet content. These results have a bearing on the functional role of the important regulator of *M. tb* chromosomal replication belonging to the LysR family of bacterial regulatory proteins, in the context of latency.

180: Bacterial toxin induced stress response in murine macrophages

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Anthrax toxin, produced by gram positive bacterium *Bacillus anthracis* comprises a set of three proteins, Protective Antigen (PA), Lethal Factor (LF) and Edema Factor (EF). Individually these proteins are nontoxic but in combination cause shock like symptoms and death. Lethal toxin (PA in combination with LF) has been shown to induce apoptosis by cleaving the amino-terminal extension of mitogen activated protein kinase (MAPK) kinases (MKKs) that activate p38 MAPKs. The intermediate events that lead to apoptosis due to anthrax toxin action are poorly understood. In this study, murine macrophage cell line J77A.1 stimulated with anthrax toxin induced the endoplasmic reticulum (ER) stress response. The changes in expression of important ER stress response genes like Hspa5 were explored by real time PCR and western blot in cells treated for varying time points with toxin. Alterations in the redox status of a cell or excessive reactive oxygen species production can cause ER stress and induce the unfolded protein response. Thus, we also explored changes in the expression and activity of various pro-oxidants and antioxidants using biochemical assays, real time PCR and western blot. The results indicated temporal increase in oxidative stress and ER stress in response to toxin treatment.

181: Predicting protein-protein interactions from metagenomics datasets

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It is now understood that even completely-sequenced genomes present only a partial snapshot of cellular inventory and processes. The bulk of processes happen at the RNA and protein interaction levels. Since interaction information of metagenome data is generally not available, people have used genome sequence to predict protein interactions and functions. However, proteins rarely act alone. They frequently assemble with other proteins to enable cellular functions. With millions of new genes identified through metagenomics studies, the task of identifying proteins, their interactions and their function, is truly enormous. We have embarked upon the mission of virtually constructing molecular networks for organisms for which genome sequence is the only available data. As a first step, we are focusing on protein-protein interactions. We hope to implement the modules in the iMetaSys workflow. The module takes input proteins as single FASTA format and outputs as simple interaction format (SIF). In addition, we hope to use network description for annotating protein

functions. Although our initial results are promising, the sheer complexity of information is likely to generate false positives. To overcome that we hope to conduct functional metagenomics experiments to reconstruct a reasonably accurate picture of molecular interactions, both within and between organisms.

182: Spo0B: A snapshot in kinase evolution

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Introduction: Spo0B is an important component of phosphorelay, the pathway involved in the initiation of sporulation in *Bacillus subtilis*. The Spo0B of *B. anthracis* is quite different from that of *B. subtilis* with only the NH2-terminal domain showing similarity between the two species. Methods: (1) Bioinformatic and phylogenetic analysis. (2) Dimerization assay of Spo0B. (3) Autophosphorylation assay of Spo0B and its mutants. (4) Acid/Base Stability Assay. (5) ATPase assay of Spo0B. (6) Phosphotransferase assay of Spo0B. (7) ATP binding to Spo0B using Isothermal Titration Calorimetry and Flourescence of tryptophan. Results: During the course of evolution Spo0B has retained the characteristic histidine kinase boxes H, N, F, G1 and G2 and has acquired nucleotide binding domains, walker A and walker B of ATPases. Due to the presence of these domains, autophosphorylation and ATPase activity was observed in Spo0B of *B. anthracis*. The thermodynamic and binding studies of Mg-ATP to Spo0B using isothermal titration calorimetry (ITC) suggested that the binding is driven by favorable entropy changes and the reaction is exothermic, with the apparent dissociation constant (Kd) equal to 0.02 mM. The value of dissociation constant (Kd = 0.05 mM) determined by the intrinsic fluorescence of trytophan of Spo0B was similar to that obtained by ITC studies. The purified Spo0B of *B. anthracis* also showed nucleoside diphosphate kinase-like activity of phosphate transfer from nucleoside triphosphate to nucleoside diphosphate. Conclusion: This is first evidence proposing Spo0B of *B. anthracis* as an enzyme with histidine kinase and ATPase activities, which may have important role to play in sporulation and pathogenesis.

183: TLR2 arg753gln and Vitamin D Receptor (VDR) gene polymorphisms in tuberculosis patients of Ahirwars and Muslim population from Uttar Pradesh, India

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Introduction: TLRs (Toll like receptors) are a family of trans-membrane receptors, which play a crucial role in recognition and presentation of antigens to adaptive immune system. Vitamin D receptor (VDR), a member of steroid hormone receptor, determines the growth of *Mycobacterium tuberculosis* in macrophage. The single nucleotide polymorphisms in these candidate genes are widely implicated in the increased susceptibility to tuberculosis. The present study was carried out in Ahirwars, a lower Hindu caste group and Muslim population group from Jhansi, Uttar Pradesh, having high prevalence of tuberculosis as appeared from the records of the District TB hospital as well as our recent survey.
Objectives: we made a preliminary analysis and calculated the frequencies of various genotypes of TLR2 and VDR genes in cases and control subjects of both populations in order to identify the most susceptible genotype for both the candidate genes, if any, which may be associated with the increased susceptibility of these groups to mycobacterial infection.
Methods: About 100 blood samples each were collected from both the groups (total, $n = 200$) including cases and controls after well informed and written consent of the participants. Arg753gln of TLR2 and Fok1, Apa1 polymorphisms of VDR gene were studied by ARMS-PCR and PCR-RFLP method, respectively at the Centre for Genomics, JU, Gwalior.
Results: The results showed that Muslim cases and controls have high frequency (0.72) of GG-genotype in both the sexes, while in Ahirwars it is about 0.49. The GA (heterozygote) genotype was found significantly ($P > 0.005$) high in cases of both the groups. However, overall heterozygosity was high in case subjects of Muslim group and Ahirwars than controls as against an earlier observation made in Turkish populations. The frequency of homozygous AA genotype was 0.05 in male controls of Muslim group. On the other hand, the frequency of FF genotype of VDR was 0.84 in male cases of Ahirwars and 'ff' homozygote genotype had a frequency of 0.1 in female cases.
Inferences: The GA genotype of TLR2 arg753gln polymorphism may have some functional association with disease which needs further study on larger sample size. The higher frequency of GG homozygotes in Muslims indicates inbreeding in this group. Further, the male cases with FF genotype of VDR gene may have a higher risk of developing pulmonary tuberculosis. [Financial support to PKT from DBT is gratefully acknowledged.]

184: *De novo* genome assembly of a highly virulent *Burkholderia pseudomallei* isolate assisted by genome paired-end ditag sequencing

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Burkholderia pseudomallei, the causative agent of melioidosis, infects humans and animals through direct contact with contaminated environmental samples, such as soil and water. The clinical manifestations are diverse, but severe pneumoniae and sepsis are common symptoms that render melioidosis a life-threatening disease with very high mortality. We have isolated a strain of *B. pseudomallei*, Bp22, that is highly virulent in animal models when compared to other isolates of this species, and preliminary data has indicated that genetic variations might account for the increased pathogenicity. Therefore, we sought to obtain the whole genome sequence of Bp22 for comparative analyses with previously sequenced genomes of *B. pseudomallei*.

Shotgun sequencing of genomic DNA from Bp22 was performed using massively parallel 454 sequencing (GS20, average read length 100 bp). *De novo* assembly of such relatively short reads, however, usually generates many unordered contigs. To facilitate completion of such a draft assembly, targeted PCR and capillary sequencing may be used to order contigs and fill gaps. This is nevertheless a time-consuming and expensive process and we therefore intended to develop a robust method for *de novo* genome assembly using only high-throughput sequencing data. This was achieved by a genome Paired-End diTag (gPET) sequencing approach, in which paired-end sequences were obtained through an in vitro cloning method. We generated four gPET libraries from Bp22 using DNA targets with genomic spans of 1, 2, 5 and 10 kb. The extracted gPETs were mapped to 988 contigs that were derived from assembly of shotgun reads. The number of resulting scaffolds ideally corresponds to the number of chromosomes in the studied genome. However, using single gPET libraries, a larger number of scaffolds, which were inversely correlated to the span size of the gPET library, were

obtained, partly reflecting the number and sizes of repetitive segments in the genome. By using a combination of two gPET libraries of different spans, we were able to order all contigs into two scaffolds, representing the two chromosomes of *B. pseudomallei*. Furthermore, an in-house developed algorithm was used for gap-filling using remaining shotgun reads as well as gPET reads.

In conclusion, we have developed a robust and straightforward means of *de novo* assembly of bacterial genomes. Analyses of the obtained Bp22 genome sequence will help us increase our knowledge of virulence mechanisms of this human pathogen.

185: Analysis of codon usage pattern in HIV-1genome and genes

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Human Immunodeficiency Virus (HIV), a retrovirus that belongs to the Lentivirus genus, is known to cause Acquired Immunodeficiency Syndrome (AIDS). Of the two types of HIV, type 1 is considered to be more infectious and has higher mortality rate compared to the type 2 virus. HIV-1 is known to be associated with very high mutation rates, which is an impediment to successful vaccine development and also leads to drug resistance. Positive selection is known to dominate the evolution of HIV-1 within a particular host, as has been shown experimentally by studying patients over a long duration of time. This is not the case for transmission among hosts, and neutrality is assumed to be the driving force for the inter-host evolution (Rambaut et al. 2004). No definitive study of the inter-host evolution of HIV-1 genome and its genes over a period of time has been done, which can document the presence/absence of any selection that can be used as a basis to devise methods to tackle HIV.

This study was aimed towards quantifying the evolutionary trend of HIV-1 genome and the nine genes (*env*, *gag*, *nef*, *pol*, *rev*, *tat*, *vif*, *vpr*, and *vpu*) using their base composition profile from the genomic sequences of different clades and regions. Since a distinct base composition from the host can be disadvantageous at the transcription/translation step for HIV-1, we analyzed the codon usage pattern in large number of sequences of each gene to address the issue of evolution of HIV-1 among different hosts over time. The overall base composition profile for the HIV-1 genome and its individual genes remains constant over time and across different clades. A preference for AT3 codons is seen in all the genes whereas, human primarily prefer GC rich codons. A large-scale analysis of codon usage bias over the period of 23 years for the HIV-1 genes showed that the structural genes and the regulatory genes differ in their pattern of codon usage. We found a temporal correlation in the variation for most of the regulatory genes, which indicate that translational selection may be the force that is shaping the evolution of some HIV-1 genes in inter-host transmission.

Reference:

Rambaut A, Posada D, Crandall KA, Holmes EC (2004) The causes and consequences of HIV evolution. Nat Rev Genet 5:52–61.

186: HIV-1: Identification of a novel kinase as a potential drug target

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DNA Topoisomerases are ubiquitous enzymes that resolve topological constraints of DNA like supercoiling, knotting and catenation. Topoisomerases catalyze these rearrangments through formation of transient breaks in the DNA using a conserved Tyrosine at its active site. Human Topoisomerase II is a type IIA Topoisomerase, which exists in two isoforms: (a) topoisomerase II alpha 170 kDa; and (b) topoisomerase II beta 180 kDa. The role of Topoisomerase II in HIV-1 replication is being hypothesized as there is a strong Topo II binding and cleavage sites both in HIV as well as at the upstream from integration site in the human chromosome. The phosphorylated forms of Topoisomerase II isoforms are the active forms and phosphorylation is the process of regulation of Topoiosmerase II in the above cellular process. In HIV-1 infected cells, the activity of Topoisomerase II is highly regulated, which determines the progression of the host Topoiosmerase II isoform-dependent virus replication events. The virus-associated Topoisomerase II kinase has been purified to homogeneity using ion-exchange chromatography and analysed using MALDI-TOF. The result significantly maps this kinase in the gag-pol precursor region. The different structural class of inhibitors tested against the purified protein has shown a distinct pattern of action over the two isoforms of DNA Topoisomerase II.

187: Use of mass spectrometry derived data to annotate genome of *Candida albicans*

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Genome annotation of infectious microorganisms will provide molecular signatures which will help in understanding molecular mechanisms of virulence, interaction with host, physiological adaptations within the host and thereby development of molecular diagnostics and therapeutic intervention. Even for the genomes which are completely sequenced, identification of protein coding genes remains a challenging task. High resolution mass spectrometry provides peptide data that can be used to verify a number of hypothetical proteins in an organism of interest. The assignment of peptide data can be performed by searching against non redundant protein databases which comprise of predicted and proven proteins/transcripts. Alternatively, novel proteins and exons can also be discovered by directly searching the mass spectrometry derived data against the genome. We have investigated the proteome of Candida albicans using high resolution mass spectrometry. We searched mass spectra obtained from 30 LC-MS/MS runs of Candida albicans cell homogenate against the Candida albicans protein and genome databases. Our findings show that this approach can be used to enrich and correct genome annotation and maximize protein discovery.

188: 'Codon-shuffling' method, a potential strategy to combat human pathogens

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Our lab has invented a novel approach towards protein evolution, which is called as 'codon-shuffling' method. This method can be

exploited to obtain the *de novo* libraries of novel and functional proteins. This approach entails in vitro assembly of 14 hexamer duplexes of DNA (Dicodon 'DC') each of which codes for two amino acids. These 14 DC comprise all 20 naturally occurring amino acids and can be ligated either in equal ratio to make an equimolar library or in skewed ratio to create a positively, negatively or hydrophobic library. We had shown earlier that some of such *de novo* synthesized proteins could acts as potential antibacterial in *E. coli*. Currently, our work involves the synthesis and selection of 'codon-shuffled' *de novo* proteins that bind to selected *Mycobacterium tuberculosis* protein targets. Using a versatile bacterial two-hybrid system, that entails utilization of the *Mycobacterium tuberculosis* protein targets and various 'codon-shuffled' protein libraries as bait and prey, respectively, we were able to identify proteins that bound strongly to the targets in our case the HupB protein of *Mycobacterium tuberculosis*. The protein binders were expressed in *Mycobacterium smegmatis* and shown to appreciably affect the growth in the exponential phase, a period wherein HupB is selectively expressed. Electron microscopy of the affected mycobacterium elaborated the extent of cell damage and hinted towards a cell-division defect. Our strategy is adaptable for large-scale targeting of the essential protein pool of *Mycobacterium tuberculosis* and other human pathogens.

189: Effect of IL1B promoter polymorphism on down regulation of gastrin through signalling intermediates NFKB and SMAD7

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Helicobacter pylori is a gastric pathogen that chronically infects the gastric mucosa of more than half of the world's population. Two polymorphisms −511C>T and −31C>T in the IL1B promoter have been suggested as potential susceptibility loci for Helicobacter pylori associated gastro duodenal diseases. Gastrin, one of the hormones that regulate gastric acid secretion, is involved in the pathogenesis of gastroduodenal ulcerations. The present study investigated the effect of IL1B promoter polymorphism on the transcriptional activity of the human gastrin promoter in a gastric epithelial cell line, AGS, and analyzed the underlying molecular mechanisms. Treatment of AGS with IL1B resulted in a 20-fold reduction in gastrin expression. Analysis revealed that IL1B represses gastrin through TAK1, TAB 1 that ultimately up regulates NFkB. A 40% release of IL1B mediated gastrin repression, as a result of inhibition of NFkB translocation, further suggested the presence of NFkB independent pathway. It was observed that IL1B up regulates Smad7, which inhibits gastrin expression by sixfolds. These results were also validated in vivo, where, at least a threefold lower expression of Smad7 and NFkB in *H. pylori* an infected individual with ulcer compared to infected asymptomatic individuals was observed. Analysis of the gastrin promoter suggests the involvement of repressor complexes consisting of NCOR and HDAC1 in IL1B mediated gastrin repression. The differential effect of IL1B promoter variants on its transcription and subsequent regulation on gastrin expression was also investigated. There was almost threefold increase in IL1B expression when AGS cells were transfected with −31TIL1B expression construct in comparison to −31CIL1B. The −31TIL1B induced a twofold greater repression of the gastrin luciferase activity compared to the −31CIL1B. This signaling of the variant allele driven IL1B revealed an almost 1.5-fold greater expression NFkB in the −31TIL1B transfected AGS cells. Experiments with HDAC inhibitor also suggested that at a particular dose, the repression on the gastrin promoter in −31CIL1B-transfected cells was greater than in cells transfected with −31TIL1B. The in vitro study has, therefore, successfully reflected at least part of the in vivo scenario resulting from genetic polymorphisms.

190: Protein phosphorylation and stress-dependent sigma factor regulation in *Mycobacterium tuberculosis*

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The establishment of *Mycobacterium tuberculosis* (*M. tb*) in the host and its long-term survival in non-replicating state is ensured by upregulation of virulence and stress response pathways through transcriptional reprogramming. The critical role of sigma factors in transcriptional adaptation is well established in the physiology and pathogenicity of *M. tb*. One of the alternative sigma factors in *M. tb* sigF plays a role in pathogen's infection and dormancy processes. The expression and modulation of sigma factor activity is mediated through a phosphorylation mediated cascade involving anti-sigma factors and anti-anti-sigma factors. Although anti-sigF and anti–anti-sigF proteins have been identified in *M. tb*, the phosphorylation-dephosphorylation events regulating the pathway have not been established. *M. tb* genome encodes a potential sigF regulatory gene, Rv1364c, which exhibits a multidomain architecture, in which the components of entire regulatory cascade for sigF are encoded within a single polypeptide. The comparative genomic analysis of all sequenced mycobacterial genomes and contextual information to identify Rv1364c orthologs helped us recognize a distinct pathogenic species-specific domain fusion event in the formation of Rv1364c. Sequence analysis of *M. tb* Rv1364c resulted in the prediction of various domains viz. a sensory domain, a coiled coil motif, a phosphatase (RsbU) domain, an anti-sigF (RsbW) domain and an anti–anti-sigF (RsbV) domain. The RsbU domain sequence possesses all conserved features of metal ion dependent PP2C type serine/threonine phosphatase family. On the other hand, in RsbW domain of Rv1364c, certain substitutions and deletions were observed in regions important for nucleotide binding. Another anti-sigF protein in *M. tb*, UsfX was found to have apparently even more unfavorable substitutions in G1 box and N box. We validated the predictions of in silico analysis experimentally, and observe that the RsbU domain of Rv1364c has phosphatase activity, whereas its RsbW domain and UsfX protein, despite being functional anti-sigF proteins, lack ATP binding and therefore kinase activity. Since, Rv1364c is an active phosphatase and as per our results, likely to be very specific to its substrates with regard to site and residue of phosphorylation, we speculated that some other kinase may fill this missing link in phosphorylation dependent regulation of sigF by Rv1364c and UsfX in Mycobacterium genus. The implications of these aspects will be discussed in presentation.

191: The Apicoplast genome of *Plasmodium vivax*

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Plasmodium vivax malaria is prevalent in many regions of Asia and the Latin American subcontinent, and there are a few places where *P. vivax* is transmitted exclusively. Almost 1–2 million *P. vivax* malaria cases are reported annually in India. Recent reports on severe manifestations of *P. vivax* malaria indicate the need for identification of novel drug targets to combat this disease. The plastid-like organelle (Apicoplast) in *Plasmodium* species has generated immense interest as a target for drugs against malaria. Apicoplast is a 3–4 membrane organelle, carrying various metabolic pathways and a circular DNA. We have characterized the organelle and various genes from this circular genome for the first time from *P. vivax* isolates. The genome is known to carry various genes of functional importance including ssu and lsu ribosomal RNA and tRNA genes, sufB, clpC, tufA genes, RNA Polymerase B, C and D subunit genes and various ribosomal protein genes. The presence of this organelle and its genome in parasite forms the basis of the present investigation. Various genes from the Apicoplast genome of *P. vivax* isolates were amplified, sequenced and analyzed. On comparison with the *P. falciparum* sequences variations were observed at both nucleotide and amino acid level. A comparative analysis of *P. vivax* Apicoplast genes with alleles from other *Plasmodium* species was also done. About 8–13% differences were observed between *P. vivax* and *P. falciparum* Apicoplast sequences. To colocalize the Apicoplast within *P. vivax* blood stages, peptides were designed from the *P. vivax* Apicoplast tufA gene and antibodies were raised in swiss albino mice. Apicoplast was colocalized using antibodies raised against Ef–TuA peptides in *P. vivax* infected blood smear slides obtained from the field.

192: Sequence variations within HPV16 isolates among Indian women: prediction of causal role of rare non-synonymous variations within intact isolates in cervical cancer pathogenesis

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Introduction: In India, HPV16 is detected in 50% of cervical cancer cases (CaCx) and it is the most predominant type within various populations. A fraction of the infected individuals develop the disease suggesting that multiple pathways act to mediate cervical carcinogenesis.
Objectives: To (1) examine the cumulative effect of nucleotide variations within majority of HPV16 coding and regulatory regions (LCR) as haplotypes on CaCx development; (2) re-examine the evolutionary distinct lineages of HPV16 in the light of haplotypes relevant for disease pathogenesis; and (3) identify the sequence variations biologically relevant for oncogenicity of HPV16.
Methods: We re-sequenced $\sim$6 kb of HPV16 genome relevant for transformation (LCR, E2, E5, E6, E7, L1, L2) from CaCx cases ($n = 74$) and asymptomatic controls ($n = 24$). NETWORK software was used to identify distinct phylogenetic clustering among the viral isolates. The SIFT (Sorting Intolerant from Tolerant) tool, which considers that amino acids conserved across viral species are more likely to be functionally significant, was used to predict non-synonymous (NS) variations deleterious to protein functions.
Results: Of 271 nucleotide variations, NS changes in L2 were higher ($P = 0.005$) in cases (2.67%) than controls (1.27%). There were 29 NS changes (frequency = 0.01–0.03), predicted as deleterious to protein functions. Haplotypes of 110 polymorphic variations (frequency $\geq$ 0.05) within intact viral isolates (53 CaCx cases and 21 controls) confirmed Asian American (AA, 14.86%) and European (E, 85.14%) variants, differing at 78 positions. The non-coding region between L2 and E5 had repeat variations, distinct for the viral lineages or subgroups of each lineage. The E-variants portrayed 36 haplotypes, and E-12 was most prevalent within cases (38.1%) and controls (28.57%) harboring polymorphic variations at 10 positions, compared to HPV16R. E-12 cases harbored 7 deleterious variations distributed within L1, E2, E5, and L2, and none among controls.
Conclusion: The E-12 haplotype defines the infection pool of HPV16 E variants prevalent in the population. The rare deleterious variations within E-12 cases reflect the pathogenic status that might have resulted over the common haplotype background. Such deleterious variations within genes that participate in productive infections within the E-12 haplotype background reflect that these variations might stall productive infection thus paving a way towards disease causation.

193: Variants of host adhesion and immune regulatory molecules and Plasmodium falciparum disease severity in a malaria endemic and non-endemic region of India

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The pathogenesis of *Plasmodium falciparum* infection is dependent upon the virulence of the infecting parasite and the immune states and the genetic make-up of the human host. The pathogenicity of *P. falciparum* results from its unique potential to modulate the host immune system and its ability to adhere to the host venular capillary endothelium. To investigate the contribution of host genetic factors in determining the susceptibility/resistance to *P. falciparum* malaria and to compare region-specific differences in immune responses, we conducted a case-control study in two regions of varying disease endemicity in India. Populations inhabiting regions of varying disease intensity differed in the relative measures of cytokines generated in response to *P. falciparum* infection. Multivariate cluster and step-wise discriminant analysis revealed that patients from endemic and non-endemic regions show discrete patterns of cytokine profiles suggesting that population inhabiting endemic and non-endemic regions differ in their clinical response to malaria. Among the regulatory region polymorphisms analyzed in cytokine encoding genes, the IL4 −590 and an ins/del polymorphism in the IL12 promoter were associated with protection from *P. falciparum* malaria in the endemic and non-endemic region, respectively. Additionally, four genetic variants in three adhesion molecule genes exhibited correlation with falciparum malaria. The mutant homozygote for the ICAM1 exon 6 (A/G, Gln/Lys) SNP was identified as a risk factor for severe falciparum malaria. While, the −53G/T mutation of the CD36 gene correlated with protection from severe malaria, exon 1a (T/A) SNP of the gene was associated with disease severity. Interestingly, the exon 3 (C/G, Leu/Val) PECAM1 SNP correlated differentially with falciparum malaria in regions of varying endemicity. The mutant G allele is risk factor for severe malaria in the endemic region, while an inverse correlation exists in the non-endemic region where the G allele associates with protection from falciparum malaria. Taken together, our results indicate that endemic and non-endemic populations substantially differ in their relative immune responses and allelic association profiles to *P. falciparum* malaria disease severity and highlight the importance of comparative analysis of association studies in regions of varying endemicity.

194: Higher chlamydial heat shock protein specific levels of Interferon-gamma, Interleukin-10 and Tumor Necrosis Factor-alpha at the site of infection is associated with infertility in women

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Background: The magnitude of reproductive morbidity associated with sexually transmitted *Chlamydia trachomatis* infection is enormous. *C. trachomatis* infection induces an inflammatory response that is crucial in resolving acute infection but may also play a key role in the pathogenesis of *C. trachomatis* associated infertility. The susceptibility, course and outcome of diseases are determined by environmental factors, virulence factors of the pathogen and host factors. Immune responses to chlamydial heat shock proteins (cHSP) 60 and 10 have been associated with the pathogenesis of *C. trachomatis* associated ectopic pregnancy and tubal infertility. However, these studies were restricted to the peripheral immune responses. Hence study of specific cytokine responses of mononuclear cells from the infectious site to cHSP60 and cHSP10 may elucidate the actual role of both host and pathogen factors in the cause of immunopathogenesis and the disease outcome.

Methods: Female patients ($n = 368$) attending the gynecology outpatient department of Safdarjung hospital, New Delhi were enrolled for the study and were clinically characterized into two groups; chlamydia positive fertile women ($n = 63$) and chlamydia positive infertile women ($n = 70$). Uninfected healthy women with no infertility problem were enrolled as controls ($n = 39$). cHSP60 and cHSP10 specific cytokine responses (Interferon (IFN)-gamma, Interleukin (IL)-10, Tumor Necrosis Factor (TNF)-alpha, IL-13 and IL-4) were assessed by ELISA in stimulated cervical mononuclear cell supernatants.

Results: cHSP60 and cHSP10 stimulation results in significant increase in IFN-gamma ($P = 0.006$ and $P = 0.04$, respectively) and IL-10 levels ($P = 0.04$) in infertile group as compared to fertile group. A significant cHSP60 specific increase in TNF-alpha levels ($P = 0.0008$) was observed in infertile group as compared to fertile group.

Conclusions: Our results suggest that exposure to chlamydial heat shock proteins could significantly affect mucosal immune function by increasing the release of IFN-gamma, IL-10 and TNF-alpha by cervical mononuclear cells which may consequently contribute to the immunopathogenesis associated with the infertility. Further studies on the immunogenetics of *C. trachomatis* infection will provide insight into the intriguing differences in the clinical course of infection between individuals, and could potentially lead to the identification of women at enhanced genetic risk for the development of tubal factor subfertility.

195: Development of molecular markers for the authentic detection of *Staphylococcus aureus* by polymerase chain reaction

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Staphylococcus aureus is one of the most commonly found pathogenic bacteria and is hard to eliminate from the human environment. It is responsible for many nosocomial infections, besides being the main causative agent of food intoxication by virtue of its variety of enterotoxins. Routine detection of *S. aureus* in food is usually carried out by traditional methods based on morphological and biochemical characterization. These methods are time consuming and tedious. In addition, misclassifications with automated susceptibility testing systems or commercially available latex agglutination kits have been reported by several workers. Consequently, there is a need for methods to specifically discriminate *S. aureus* from other staphylococci as quickly as possible. Microbial genomes are being sequenced at a staggering rate. Approximately, 10% of the genes of a species in a genus are unique to each organism, and we are now beginning to appreciate the genetic diversity among bacterial strains. This forms the basis for genotypic identification of microorganisms including bacterial and fungal pathogens. PCR techniques have provided increased sensitivity, allowed far more rapid processing times, and enhanced the likelihood of detecting bacterial pathogens. In the last 15 years, several detection methods have been proposed for food-borne pathogens to replace the time-consuming classical techniques. However, due to variability in selectivity of different primers, it is imperative to target new genes for the detection of *S. aureus*, so that infallible assays are developed for its detection and identification. Validation is an important requirement for the development of a PCR-based detection system. The present study is based on two new target genes fmhA and catalase, and a previously used target gene, femA. The protocols developed herein could be used for rapid and specific detection of this pathogen in environmental, clinical and food samples including milk.

196: Genetic polymorphisms in TLR1 are associated with susceptibility to leprosy

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Leprosy represents a significant health burden and remains endemic in several parts of the world. There is evidence that Toll-like receptor 1 (TLR1) mediates immune response to *Mycobacterium leprae*, the causative agent for leprosy. We hypothesize that genetic polymorphisms in the region are associated with susceptibility to leprosy. Using Sequenom® MassArray MALDI-TOF primer extension assay, we have genotyped 15 single nucleotide polymorphisms (SNPs) in the TLR 10-6-1 gene cluster in Indian samples. Strong evidence of association was obtained at a non-synonymous SNP (rs5743618) in TLR1 where the G allele was associated with significant protection in the case-control samples (OR = 0.41, $P = 0.0000056$). Significant associations were also observed for other SNPs in the region. In conclusion, we have demonstrated that genetic polymorphisms in the TLR 10-6-1 gene cluster are strongly associated with susceptibility to

leprosy, and our results suggested that TLR1 may play a crucial role in the immunity against leprosy.

197: Genome-wide transcription analysis of interaction between the human macrophage and *Mycobacterium tuberculosis* during concurrent drug administration by conventional and novel methods

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Virulent strains of *Mycobacterium tuberculosis* (MTB) induce 'alternative activation' of the host macrophage. Studies using inhalable microparticles with or without a payload of anti-tubreculosis drugs have indicated that such microparticles can rescue MTB-infected macrophages from alternative activation and induce oxidative radicals, Th1 cytokines and apoptosis. A genome-wide transcription analysis (Affymetrix HG-U133 Plus 2.0 DNA microarray) of THP-1 derived macrophages was undertaken after exposing them to infection with 10 MOI of MTB H37Rv at 0, 12 and 24 h post infection. Parallel groups were treated with drugs in solution, drug-containing microparticles or blank microparticles. About 1,500 genes were differentially upregulated and about 500 genes differentially downregulated in response to various modes of treatment. Variations were also observed in the kinetics of gene expression. Cluster analysis indicated activation of several pathways related to innate immune response (cytokines, chemokines, receptors and ligands), apoptosis, cytoskeleton and membrane remodeling, general metabolism and general housekeeping. Some of these results were validated at the functional level, by studying enzyme activities, concentrations and time-courses of effector molecules and rates/extents of apoptosis. The results compared well with published reports on transcription response of human macrophages to MTB infection. These results permitted a novel analysis of tripartite interaction between host, pathogen and drug. Such analysis is important from an appreciation of the fact that concurrent chemotherapy is the norm in tuberculosis, but the outcome of chemotherapy is likely to be significantly affected by the contribution of the host response to both infection and drug treatment. Our data provide a basis for considering the relative contributions of the macrophage response to infection and drug treatment. These data also indicate the ability of our proposed drug delivery system to induce beneficial host responses. Finally, the data offer insight into manifestations of drug toxicity in the host cell, and its possible impact on the host response.