FOCIS Associate Member Symposium

by

The Indian Immunology Society

Targeting Immune Response: Synergistic Therapeutic Strategies

Wednesday June 20, 8.00AM to 10.00AM

Chairs

Nibhriti Das
Sr. Consultant & HOD – Biochemistry, Director, Department of Lab Medicine, Nayati Multi Super Specialty Hospital, India

Taruna Madan
Department of Innate Immunity, ICMR-National Institute for Research in Reproductive Health, India

TIME	SPEAKER, AFFILIATION AND TITLE
8.00 AM	**Insight into the Indian Immunology Society** *Dr. Alpana Sharma, Treasurer-IIS, Department of Biochemistry, All India Institute of Medical Sciences, India*
8.10	**Pro and antitumor functions of human gamma delta T cells: Implications in cancer immunotherapy** *Dr. Shubhada Chiplunkar, Director, Advanced Centre for Treatment, Research & Education in Cancer, India*
8.25	**The clinical relevance of HLA and non-classical HLA genes in Transplantation** *Dr. Uma Kanga, Department of Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, India*
8.40	**Realm of innate immune cells and chemokine receptors in Pemphigus vulgaris: plausible therapeutic targets** *Dr. Alpana Sharma, Department of Biochemistry, All India Institute of Medical Sciences, India*
8.55	**Role of vitamin D in innate immunity modulation based therapeutic targets in pulmonary TB** *Dr. Archana Singh, Dept. of Biochemistry, All India Institute of Medical Sciences, India*
9.10	**TLR4 mediated immunomodulation in tuberculosis: An alternative approach to combat TB** *Dr. Pramod Gupta, Radiation Medicine Centre, Bhabha Atomic Research Centre, India*
9.25	**Regression equations in scrutinizing key laboratory markers for assessing the disease severity of rheumatoid arthritis - RA blood-based disease activity score** *Dr. Archana Bhatnagar, Department of Biochemistry, Panjab University, India*
9.35	**Genital Mucosal proteome of HIV serodiscordant couples: A key to factors inhibiting sexual transmission of HIV** *Dr. Taruna Madan (Co-Convener), Department of Innate Immunity, ICMR-National Institute for Research in Reproductive Health, India*
9.45	**SP-D, guarding the vaginal mucosal barrier against HIV-1** *Dr. Hrishikesh Pandit, National Cancer Institute, National Institutes of Health, MD, USA*
9.55	**Highlights** *Dr. Taruna Madan (Co-Convener), Department of Innate Immunity, ICMR-National Institute for Research in Reproductive Health, India*

Abstracts available at http://www.indianimmunology.org/FOCIS%202018%20schedule.pdf

Pro and antitumor functions of human gamma delta T cells: Implications in cancer immunotherapy

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During the past decade anti-cancer immunotherapy has evolved from a promising therapeutic option to robust reality that has changed treatment outcomes. Gamma delta T cells differ from conventional αβ T cells with regard to T cell receptor repertoire, antigens recognized, tissue localization and effector functions. Human Vγ9Vδ2 T cells recognize metabolites that are overproduced by transformed cells such as isopentenyl pyrophosphate (IPP) and E-4-hydroxy-3-methyl-but-2-eryl pyrophosphate (HMBPP). Aminobisphosphonates greatly enhance tumor recognition of Vγ9Vδ2 T cells by inhibiting the intracellular farnesyl pyrophosphate synthase enzyme resulting in increase in endogenous IPP. Upon antigen activation, γδ T cells showed increased expression of notch receptor, notch ligands and EZH2 (methyltransferase). EZH2 regulates the notch receptor expression through inhibition of notch signaling suppressors. Inhibition of Notch signaling in γδ T cells leads to decrease in the TCR driven expression of transcription factors Eomes and Tbet, which are essential for cytotoxic effector functions. Studies from our lab also highlighted the pro-tumor functions of a subset of γδ T cells. In particular, Tγδ17 subset is capable of recruiting immunosuppressive myeloid populations, inhibiting antitumor responses, and enhancing angiogenesis, thus promoting cancer progression. Adoptive transfer of phosphoantigen activated γδ T cells or co-administration with Aminobisphosphonates /cytokines /monoclonal antibodies appears to be a promising approach for cancer immunotherapy. A comprehensive understanding of the pro and anti-tumor functions of γδ T cells and the molecular interactions of key immune regulators would provide an impetus to bring this modality of treatment from bench to bench side.

The clinical relevance of classical HLA, non-classical HLA and non-HLA genes in Transplantation

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Degree of histocompatibility between the recipient and donor impacts the overall survival of recipients undergoing transplantation. Matching for classical HLA class I (A,B,C) and class II (-DRB, -DQ) genes reduces the alloreactivity and improves the transplant outcome. HLA-G, a non-classical HLA-class I molecule interacts with receptors ILT-2/ILT-4/KIR2DL4 on immune cells and creates a tolerogenic microenvironment. Various miRNAs interact with the polymorphic residues in HLA-G 3'UTR region and control HLA-G expression. HLA proteins also interact with Killer Immunoglobulin like Receptors (KIRs) on NK cells. In HSCT settings, these interactions impact the transplant outcome. The HLA-G 3'UTR exon 8 polymorphisms {14bp insertion/deletion (ins/del) and SNPs} were investigated. The soluble HLA-G levels were measured at pre-transplant, at several times post transplant and at graft versus host disease (GVHD) day/ kidney rejection day. Additionally; in HSCT setting, the donor KIR genotype and the recipient HLA ligand groups C1 (Asparagine 80), C2 (Lysine 80) and HLA-Bw4 were evaluated. In this study cohort, although the 14bp ins/del genotype was predominant among controls, the sHLA-G levels were highest among those carrying the 14bp ins/ins genotype. The sHLA-G levels were higher in individuals with 14bp-INS linked haplotypes UTR2 and UTR4. Lack of C1 group ligands for donor KIRs appeared to increase the risk of acute GvHD manifestation. Presence of Bw4-80(T) allele appeared to reduce the GvHD risk. We conclude that, in recipients undergoing transplants, the HLA-G 3'UTR genotyping, serial monitoring of sHLA-G levels in serum/plasma and KIR-HLA interactions appear to be of significant clinical relevance HLA-G molecule can possibly serve as a biomarker to predict transplant outcome.

Exploring the realm of innate immune cells and chemokine receptors as the potential target for PV

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Pemphigus vulgaris (PV) is a life threatening and severe autoimmune disease affecting skin and mucosa. DCs are most crucial antigen presenting cells, playing role in immune response, tolerance and autoimmunity. In our maiden study, we have observed significantly decreased frequency of Myeloid DC (mDC) & Plasmacytoid DC (pDC) in circulation of PV patients as compared to controls. The relative mRNA expression CD40 & CD80 was significantly increased, while a significant decline in inhibitory markers (PSGL1 & ILT3) was observed in DCs from PV patients. We have confirmed the localization of DCs in the perilesional skin of patients and also observed increase in the inflammatory cytokines associated with DCs. These findings further strengthen the defect in functional status of DCs along with the co-stimulatory molecules that might be contributing in the pathogenesis of PV. γδ-T- cells play significant role in the immune surveillance at the epithelial surface. In our study, we have found increased frequency of γδ-T- cells along with IFN-γ suggesting a Th1 polarization pattern. We have observed dual cytokine producing (IFN-γ, IL-4) and IL-17 secreting γδ-T- cells in PV patients suggesting the plasticity of these versatile immune cells. Relative mRNA expression levels of both scavenger receptors CD36 and CD163 were higher in PV patients indicating the involvement of these receptors in this disease. DCs, γδ-T- cells and the scavenger receptors and their significant contribution to the immunopathogenesis of PV demand to study their potential to develop novel therapeutic targets in this disease.

Role of vitamin D in innate immunity modulation based therapeutic targets in pulmonary Tuberculosis

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Vitamin D, its transport protein vitamin D binding protein (VDBP) and vitamin D receptor (VDR), may play a role in altering the host defense against Mtb via production of cathelicidin (antimicrobial peptide) and regulating the expression of inducible nitric oxide synthase (iNOS) required for production of bactericidal Nitric oxide (NO). In the present study, levels of Vitamin D, NO and their associated molecules were evaluated in 100 active TB patients, 75 household contacts and 70 healthy controls. VDR and iNOS mRNA levels were found to be lower in active TB group compared to household contacts and healthy controls (P=0.0001 and 0.005 respectively). Though insignificant, expression of VDBP mRNA was lower in active TB group as compared to household contact and control groups. The serum levels of Vitamin D were also found to be lower in active TB group compared to healthy controls (P =0.001). Levels of cathelicidin and NO were higher in patient group as compared to other groups (p=0.01 and 0.5 respectively). However, the expression of VDR and iNOS and levels of vitamin D was significantly (P< 0.05) higher in household contacts compared to both active TB and healthy control groups. Our observations suggest that vitamin D might have a protective role against TB which prevents activation of the disease. Decreased vitamin D levels could be implicated in disease progression. Supplementation of vitamin D and arginine alone in household contacts of TB and as an adjuvant therapy along with ATT in active TB should be evaluated to assess therapeutic potential.

TLR4 mediated immunomodulation in tuberculosis: An alternative approach to combat TB

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Frequent therapeutic failures and emergence of drug resistant strains has necessitated for the development of better approaches for management of Tuberculosis (TB) and modulation of host immune responses offers an attractive novel approach to control the pathogen. In the present study, the immunomodulatory effects of G1-4A, a polysaccharide derived from the Indian medicinal plant, Tinosporacordifolia, have been investigated in *in-vitro* and aerosol mouse models of MTB infection. G1-4A treatment of MTB infected RAW264.7 macrophages significantly induced the surface expression of MHC-II and CD-86 molecules, secretion of proinflammatory cytokines (TNF-α, IL-β, IL-6, IL-12, IFN-γ) and nitric oxide leading to reduced intracellular survival of both drug sensitive (H37Rv) as well as multi drug resistant strains (Beijing and LAM) of MTB which was partially attributed to G1-4A induced NO production in TLR4-MyD88 dependent manner. Similarly, bacillary burden was significantly reduced in the lungs of MTB infected BALB/c mice treated with G1-4A, with simultaneous up-regulation of the expression of TNF-α, INF-γ and NOS2 in the mouse lung along with increased levels of Th1 cytokines like IFN-γ, IL-12 and decreased levels of Th2 cytokine like IL-4 in the serum. Furthermore, combination of G1-4A with Isoniazid (INH) exhibited better protection against MTB compared to that due to INH or G1-4A alone, suggesting its potential as adjunct therapy. Our results demonstrate that modulation of host immune responses by G1-4A might improve the therapeutic efficacy of existing anti-tubercular drugs and provide an attractive strategy for the development of alternative therapies to control tuberculosis.

Regression equations in scrutinizing key laboratory markers for assessing the disease severity of rheumatoid arthritis - RA blood-based disease activity score

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The heterogeneous nature of rheumatoid arthritis (RA) and ambiguous pathogenesis made it difficult for extrapolating the disease activity in terms of laboratory markers. The present study looks into the role of oxidative stress and immunobiology of NK and NKT cells in designing a mathematical model for assessing disease severity in RA patients. A disease activity score based on objective laboratory markers will eliminate the subjective nature of current gold standards being used in practice and trials. Fifty RA patients were recruited from the Rheumatology Clinic of PGIMER Chandigarh. Fifty age and sex-matched healthy volunteers were also enrolled. The biochemical parameters investigated in serum samples were Lipid Peroxidation, Reduced Glutathione, Catalase, Superoxide dismutase, Glutathione peroxidase, IL-18, and TNF-α. NK and NKT cells related intracellular parameters (DNA damage, caspase-3, perforin, granzyme A and B, IL-4, IFN-γ and IL-8 expression) were measured using multicolor flow cytometer. The mathematical model equation was developed for predicting RA blood-based disease activity score (RABBDAS) using multiple linear regression analysis. The oxidative balance was deregulated in RA patients. On scrutinizing laboratory markers using regression equation employing significance ($p<0.005$), Granzyme A, perforin and IL-8 were found to be independently associated with RABBDAS as per the following equation:

$RABBDAS = -1.115 + 0.054(\%GranA^{+}NK) + 0.032(\%Perf^{+}NKT) + 0.14\ \%(IL\text{-}8^{+}NKT)$ ($r2 = 0.95$)

There is a state of profound oxidative stress in RA patients. Immunobiology of NK and NKT cells were severely compromised. The current disease activity score needs to be validated in trials.

Genital Mucosal proteome of HIV serodiscordant couples: A key to factors inhibiting sexual transmission of HIV

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Evidence from HIV-1 highly exposed sero-negative (ESN) individuals, such as, sex workers and serodiscordant couples, suggests that mucosal factors in the genital tract, the first site of contact for the virus, can preventviral transmission. Identifying naturally occurring anti-HIV factors is critical for the development of effective preventative strategies. To the best of our knowledge, this is the first report on mapping of genital mucosal proteomes of HIV serodiscordant couples. A total of 112 study participants were recruited in three groups, [HIV infected, males (30) females (30); Serodiscordant males (15) females (17); and Controls males (30) females (30)]. Quantitative iTRAQ analyses was carried out on Cervicovaginal lavage (CVL) and seminal plasma (SP) . A total of 1163 non redundant proteins with 39,083 PSMs were identified from the CVL and 432 proteins were differentially expressed. Major functional categories of differentially expressed proteins in the CVL were cytoskeletal proteins of mucosal epithelial barrier, proteases and protease inhibitors, inflammation associated molecules, and virus neutralising proteins. Importantly, upregulated levels of two of the protease inhibitors, Elafin and Cystatin B, in the CVL of serodiscordant females were validated by ELISA. A total of 1477 non redundant proteins with71,561 PSMs were identified from the SP and 387 proteins were differentially expressed. Major functional categories of differentially expressed proteins in the SP were Proteasome complexes , cytokine signaling , lysozyme activity, Complement pathway and Neutrophil degranulation. Some of these potential candidates may be explored for development of comprehensive vaginal/rectal microbicide gels to simulate the proteome prevalent in serodiscordant females/ males.

SP-D, guarding the vaginal mucosal barrier against HIV-1

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It is important to understand the mucosal gatekeeping mechanisms and early innate immune response to develop an effective preventive strategy against sexual transmission of HIV-1. We describe a novel strategy to test candidate microbicides*ex vivo* using multilayered organotypic vaginal-ectocervical epithelia (EpiVaginal) tissue and activated human PBMCs. Using the tool we report a global gene signature of HIV-1 challenged EpiVaginal tissues and major determinants of sexual transmission were genes regulating inflammation, tight junctions and cytoskeleton framework. Surfactant Protein D, a pattern recognition molecule and regulator of innate and adaptive immune responses, is naturally secreted by the vaginal epithelial cells under hormonal control. In view of our earlier findings that a recombinant fragment of human SP-D (rfhSP-D) possesses a potent and broad-spectrum anti-HIV-1 activity, we validated its efficacy in our model. HIV-1 transfer to the PBMCs in basal chamber were significantly inhibited by rfhSP-D and EpiVaginal tissues showed a remarkable reversal of HIV-1 induced gene signature in presence of rfhSP-D. Supernatants of rfhSP-D conditioned EpiVaginal tissue did not enhance susceptibility of target cells to HIV-1. rfhSP-D did not alter baseline viability, NF-κB activation and levels of immune mediators in the treated EpiVaginal tissues. Importantly, no adverse effects were observed on *Lactobacilli in vitro* and in the rabbit vaginal irritation model establishing the safety of repeated vaginal application of rfhSP-D. The*ex vivo* model of vaginal HIV-1 transmission revealed global gene signatures facilitating and preventing viral transmission. Study established that rfhSP-D effectively retains its anti-HIV activity and maintains immune-physiology at mucosal sites highlighting its potential for a preventive microbicide.