

LATE BREAKERS

LATE-1 - Poster --POSTER SESSION A (MONDAY, AUGUST 2)

Clinical Occupation is Associated with Higher Msg Antibody Levels over Time.
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Rationale. In the absence of a method to culture *Pneumocystis jirovecii*, seroepidemiologic studies have been critical to better understanding the organism's mode of transmission. Previously, we found in health care workers (HCW) that clinical occupation was associated with higher antibody levels to MsgC1 [Tipirneni et al. Emerg Infect Dis 2009] and with changes in MsgC1 levels over time. These findings were consistent with transmission of *Pneumocystis* within the hospital setting. In the present study, we included different Msg fragments (MsgA, MsgB, MsgC3, MsgC8, and MsgC9). The goal of this study was to compare antibody responses to these Msg fragments between clinical and non-clinical staff at San Francisco General Hospital (SFGH). Methods. We conducted a longitudinal study of 115 HCW in clinical and non-clinical occupations at SFGH. Serum specimens were collected at baseline and quarterly for one year. The serum specimens were sent to the University of Cincinnati where ELISA was used to measure serum antibodies to the various Msg fragments. Since some antibody levels were censored to "1", Tobit regression model adjusting for multiple observations on the same subject was used to estimate and compare the mean antibody responses between staff in clinical and non-clinical occupations. Results. We found that HCW in clinical occupations had significantly higher antibody responses to MsgC1, MsgC3, and MsgC8 compared to staff in non-clinical occupations (p=0.002, 0.05, 0.01, respectively). Staff in clinical occupations also had higher antibody responses to MsgC9 than non-clinical staff, but the difference was not significant. In contrast, non-clinical staff developed higher antibody responses to MsgA and MsgB compared to clinical staff, but the difference was not significant. Conclusion. Health care workers in clinical occupations had higher MsgC antibody responses over time than health care workers in non-clinical occupations. This suggests that *Pneumocystis* is transmitted via person-to-person exposure.

LATE 2. -poster - POSTER SESSION A (MONDAY, AUGUST 2)

The Role of CD₄⁺ T Cell Subsets in the Clearance of *Pneumocystis murina* Pneumonia in Mice. MINGQUAN ZHENG, MICHAEL RIPPLE, AMY C. YU, DAVID RICKS, JAY K. KOLLS. Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA 70112.

Rationale: Pneumonia caused by *Pneumocystis* species (PcP) remains a serious complication in the immunocompromised host. Previous work on the murine model has shown that host defense against *P. murina* infection is critically dependent upon CD₄⁺ T cells. Following activation by antigen-presenting cells (APCs) and a complex network of cytokine signals, naïve T cells differentiate into effector CD₄⁺ T cells with three subsets of T helpers (Th). Th1 effectors produce IFN-gamma and regulate cellular immunity against intracellular infections. Th2 cells produce interleukin (IL)-4, IL-5 and IL-13 and mediate humoral immunity against parasite infections. Th17 cells produce IL-17a, IL-17F, and IL22 for tissue inflammation and clearance of certain extracellular pathogens. However the role of specific T-cell subsets that mediate host resistance against *P. murina* infection remains unclear. Therefore, to better understand the CD₄⁺ T cell requirement for host resistance to *P. murina*, we used a series of cytokine and STAT transcription factor knockout (KO) mice to determine the role of Th1, Th2, and Th17 cells in *P. murina* infection. **Methods:** Different KO mice were infected with *P. murina*. At day14 and 28, mice were sacrificed. Serum, bronchoalveolar lavage fluid (BALF), lung tissue, and lung lymph nodes (LD) were collected. LN and lung tissue cells were analyzed for IFN-gamma, IL-5, and IL17 by ELISpot and intracellular cytokine staining. Total lung RNA were analyzed for *P. murina* burden by real-time PCR. Cytokines in BALF and cell culture supernatants were measured using bio-Plex. **Results:** IFN α R KO mice had intact Th2 and Th17 responses but significantly reduced Th1 response to PC. Stat4 KO, Stat6 KO and Stat4/Stat6 KO all were resistant to *P. murina* compared to the positive control Rag2 KO mice. Stat4 was required for Th1 responses in lymph node cells and Stat6 was required for Th2 response. Th17 response was intact and slightly elevated in Stat4/Stat6 double KO mice. Stat3 expression was required in CD₄⁺ T cells for *P. murina* Th17 responses. IL-22 KO mice showed increased *P. murina* burdens in the lung. Although IL-17 RA mice cleared the infection, IL-17RA crossed to IL-22 KO mice had higher *P. murina* burden compared to IL-22 KO mice alone. **Conclusion:** IL-23 is required for *P. murina* Th17 responses but Th1 immunity is independent of IL-12 and IL-23 but dependent on IFN α R. Th17 cytokines are required for response to *P. murina* infection. Individual Stat mutants clear PcP.

LATE 3. poster - POSTER SESSION B (WEDNESDAY, AUGUST 4)

Serologic Responses to *Pneumocystis* Proteins in Human Immunodeficiency Virus Patients With and Without *Pneumocystis jirovecii* Infection. MATTHEW R. GINGO,¹ LORRIE LUCHT,¹ KIERAN R. DALY,² KPANDJA DJAWE,² FRANK J. PALELLA,³ ALISON G ABRAHAM,⁴ JAY H. BREAM,⁴ MALLORY D. WITT,⁵ LAWRENCE A. KINGSLEY,¹ KAREN A. NORRIS,¹ PETER D. WALZER,² ALISON MORRIS¹; ¹University of Pittsburgh, Pittsburgh, ²University of Cincinnati, Cincinnati, ³Northwestern University, Chicago, ⁴ Johns Hopkins, Baltimore, ⁵University of California Los Angeles, Los Angeles, USA.

Background: Immune responses to *Pneumocystis jirovecii* are not well-understood in patients with human immunodeficiency virus (HIV), but antibody responses to different proteins may be useful as a marker of *Pneumocystis* risk or presence of *Pneumocystis* pneumonia (PcP). Methods: Enzyme-linked immunosorbent assays of antibodies to recombinant *Pneumocystis* proteins of major surface glycoprotein (Msg) fragments (MsgC1, C3, C8, and C9) and of antibody titers to recombinant kexin protein (KEX1) were performed on three sequential serum samples prior to and three samples after first AIDS-defining illness from Multicenter AIDS Cohort Study participants and compared between those who had PcP or a non-PcP AIDS-defining illness. Results: Fifty-four participants had PcP and 47 had a non-PcP AIDS-defining illness. IgG levels to MsgC fragments were similar between groups prior to the first AIDS-defining illness, but the PcP group had higher levels of IgG to MsgC9 (median units/ml 50.2 vs. 22.2, $P=0.05$) post-illness. Participants who had PcP were more likely to have an increase in MsgC3 [odds ratio (OR) 3.9; confidence interval (CI) 95% 1.3-11.5, $P=0.02$], MsgC8 (OR 5.5; 95% 2.0-15.0; $P=0.001$), and MsgC9 (OR 4.0; CI 95% 1.5-11.1; $P=0.007$). The PcP group was more likely to have low KEX1 IgG prior to development of PcP (OR 3.6; CI 95% 1.0-12.6; $P=0.05$) independent of CD4 cell count and to have an increase in high IgG titers to KEX1 after PcP. Conclusion: HIV-infected individuals develop immune responses to both Msg and kexin proteins after PcP. Low KEX1 IgG titers may be a novel marker of future PcP risk independent of CD4 cell count.

LATE 4 - platform - (replaces PL37, which has been moved as poster presentation L4 on Wednesday, Aug. 4)

new PL37

Induction of Unfolded Protein Response and Cell Death Pathway in the *Pneumocystis* Infected Lung. SALIM MERALI, Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 1914.

Endoplasmic reticulum (ER) stress occurs when the capacity to fold, modify and transport proteins cannot keep pace with protein synthesis. ER stress is sensed by a triad of specialized proteins located in the ER membrane (PERK [protein kinase-like endoplasmic reticulum kinase], ATF6 [activating transcription factor 6], IRE1 [inositol requiring enzyme-1]), which monitor protein folding. When folding is inadequate, this triad triggers the “unfolded protein response” (UPR). Protein folding within the ER involves resident chaperones (GRP78, calreticulin, calnexin, GRP94, GRP170) and a foldase/isomerase (PDI [protein disulfide isomerase]). One aspect of the UPR is up-regulation of GRP78, calreticulin, and PDI to compensate for the excess of unfolded proteins. Another aspect is activation of downstream signaling pathways that lead to up-regulation of transcription factors such as Nrf2 (nuclear factor erythroid 2 related factor 2) that increase expression of proteins involved in anti-oxidant defense. UPR is generally protective, however when ER stress is sufficiently severe and protein homeostasis cannot be restored, UPR initiates apoptosis by up-regulating expression of the pro-apoptotic transcription factor CHOP (CCAAT/-enhancer-binding protein homologous protein). We find UPR to be involved in the complex interaction between *Pneumocystis* and its host. Using two dimensional gel electrophoresis coupled with MALDI/TOF proteomics, we analyzed lungs of rats that developed *Pneumocystis* pneumonia (n=3) and compared the results to those from animals treated exactly the same but did not develop *Pneumocystis* pneumonia (n=3). Analysis of the gels revealed 22 differentially expressed protein spots; however, MALDI/TOF data showed these to represent only 16 unique proteins. The extra spots are presumably due to isoforms or post-translational modifications. Of the 16 unique proteins, 2 were found only in infected lungs, 10 in both but up-regulated in infected lungs, and 4 in both but down-regulated in infected lungs. These 16 proteins were categorized into the following groups: UPR related (GRP 78, calreticulin, PDI; all up-regulated), antioxidants/cell stress response (4; all up-regulated), structural (4; some up and some down) and miscellaneous (5; some up and some down). The up-regulation of GRP 78 was confirmed by Western blot and increases in 2 other UPR-related proteins were also detected by Western blots (CHOP, Nrf-2). Collectively, these results suggest PCP induces UPR in the lung leading to CHOP-activated apoptosis.