are known to trigger cellular TLR2 signaling response and cytokine production that seems to inversely correlate to virulence.

**Objective:** We sought to investigate the role of SOCS-1 and -3 in pulmonary inflammation and in response to mycobacteria in vitro.

**Methods:** Human PMBCs, monocytes and alveolar macrophages (AM) were obtained through standard protocols from healthy individuals and patients with pulmonary disease due to non-tuberculous mycobacteria (NTM). Cells were stimulated in vitro with NTM (M. avium, ATCC 35717 and M. abscessus, ATCC 19977) for a period between 1-20 hours. Transcriptional response was assayed by real time PCR and protein detected by ELISA, Western blot and confocal microscopy evaluation.

**Results:** Kinetic experiments performed both on purified monocytes and AM showed induction of TNF-α after 20 hour culture by both mycobacteria and the early expression of SOCS-1 (8.6±1.3 fold induction compared to unstimulated cells) and SOCS-3 (6.2±2.0) induced by M. avium as compared to M. abscessus. Evaluation of such mediators on AM obtained from patients with pulmonary NTM infection confirmed mycobacteria-induced expression in the context of chronic disease (TNF-α, 4.940pg/ml; SOCS-1, 4 fold induction; SOCS-3, 4.3 fold). In order to determine whether knock down of SOCS-1 gene interfered with mycobacteria-induced response, we performed experiments using short-interfering RNA (siRNA) which led to higher expression (~50%) of TNF-α mRNA and protein in vitro.

**Conclusion:** The data suggest that mycobacteria-induced TNF-α production through TLR activation is partially regulated by SOCS. Moreover, our work indicates that different mycobacteria species elicit differential induction and activation of cellular effector mechanisms which may have correlates in virulence, pathogenesis, and clinical course of disease.

6 Mutation 1623_1624delGCinsTT and IL-12Rb1 Deficiency: A Mutational Founder Effect on the Most Frequently Affected Gene for Mendelian Susceptibility to Mycobacterial Disease

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**Background:** IL-12Rb1 deficiency is the most common genetic etiology of Mendelian susceptibility to Mycobacterial Disease (MSMD). Known mutations affecting the IL12Rb1 gene are recessive and associated to the abolition of the response to both IL-12 and IL-23. No studies on recurrent mutations have been reported so far. Mutation 1623_1624delGCinsTT was described in 4 families (1 from Germany, 1 from Cyprus, 1 from France and 1 from Belgium). However, this same mutation was found in an unexpectedly high proportion among IL-12Rb1 deficient patients in Argentina: 6 (3 homozygous and 3 heterozygous) out of 7 affected individuals from 7 unrelated families carried this particular mutation.

**Objective:** To determine whether IL-12Rb1, 1623_1624delGCinsTT mutation represents a DNA mutational hotspot, or a founder effect where all mutants are identical by descent.

**Materials and Methods:** Thirty-four polymorphic markers in chromosome 19, internal or proximal to the IL-12Rb1 gene, were studied by direct sequencing, restriction fragment length polymorphism or microsatellite analysis in the Argentinian and the Belgian patients carrying mutation 1623_1624delGCinsTT, in order to determine if there was an haplotype associated to this mutation. An in-house modified method for estimating the age of the most recent common ancestor carrying mutation 1623_1624delGCinsTT, and based on Génin et al. likelihood-based method, was applied. Two highly polymorphic markers were also studied in 100 normal chromosomes.

**Results:** A common haplotype was shared by all chromosomes carrying mutation 1623_1624delGCinsTT, whereas it was not detected on any of the control chromosomes. The age of mutation 1623_1624delGCinsTT was estimated in 22 generations (CI 95% 8-57) (1 generation 4-5 years).

**Conclusions:** Mutation 1623_1624delGCinsTT represents a founder effect involving IL-12Rb1, the most frequently affected gene on patients with MSMD. Our calculations indicate this mutation arose 550 years ago (CI95% 200-1425), approximately by the time Spaniards’ initiated colonization of the Americas. The reason(s) behind the persistency of this mutation across multiple generations, or if it confers any type of selective advantage has yet to be established.

7 Role of Innate Immunity in Viral Pathogenesis

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**Background:** The initial interactions between host and virus are critical in determining the ultimate outcome of the infection. These interactions include the ability of cells to engulf and eliminate viruses prior to spread. Cells encounter viral antigens: 1) On the surface (after initial attachment), 2) in endosomal compartments (after viral entry), and in the cytoplasm (after uncoating and exposure of the nucleic acid). Whether a virus causes hemorrhagic fever and shock (Lymphocytic Choriomeningitis Virus - LCMV and other arenaviruses) or encephalitis (herpes simplex virus - HSV) depends on the innate immune response to the virus.

**Objective:** To define the components of the innate immune responses to LCMV and HSV.

**Methods:** Human peripheral blood cells and mouse macrophages were challenged with live or uv inactivated LCMV or HSV. Levels of interferon and inflammatory cytokines were measured in cell supernatants as well as in the serum of wild type or TLR knockout mice.

**Results:** LCMV induced production of inflammatory cytokines is dependent upon the interaction of virus with Toll like receptor 2 (TLR2), a cell surface pattern recognition protein. The production of interferon by LCMV is regulated both by TLR2 (in vivo) as well as by cytoplasmic helicases (in vivo). The cytokine response to HSV (which determines whether there are symptoms of encephalitis) is
regulated by both surface TLR2 and endosomal TLR9, and may also involve a cytoplasmic DNA sensor. The adapter protein MyD88 has a critical role in defining the adaptive immune response.

Conclusions: Viruses induce multiple pathways of innate immunity. The production of interferon and cytokines is regulated at a critical level. The adapter protein MyD88 has a critical role in defining the adaptive immune response.

8 Successful Umbilical Cord Blood Transplantation after Treatment of Rhizopus Infection in an Infant with Hemophagocytic Lymphohistiocytosis

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Background and Material: Three month old previously normal infant presented with sepsis syndrome with fever, DIC, hepatospleno-megaly, pancytopenia diagnosed with Hemophagocytic Lymphohistiocytosis (HLH) with H-MUNC mutation and no natural killer cell function. His siblings and parents were heterozygous for this mutation.

Methods and Results: After stabilization in the ICU, his initial chemotherapy was based on HLH 2004 protocol consisting of steroids, cyclosporine and etoposide. Ten days into this therapy a dark discolored painful lesion with a necrotic center was noticed on his left anterior thigh. Emergent excision biopsy revealed hyphal forms consistent with zygomycosis, with culture being positive for Rhizopus species resistant to Amphotericin B, Caspofungin, Itraconazole, Posaconazole and Voriconazole (MIC = >32 mcg/ml). Daily granulocyte transfusions, combination antifungal therapy with caspofungin and lipid formulation of amphotericin B was given for several weeks. Repeated debridement and local infiltration of lipid formulation of amphotericin B of the wound (see figure) was done until margins were free of hyphal elements. The wound was allowed to heal by secondary intention following this he was transplanted with umbilical cord blood at the University of Minnesota. He had successful engraftment but experienced renal failure, veno-occlusive disease and respiratory problems with subsequent recovery. There was no relapse of zygomycosis. The patient is now 250 days post transplant and one year has elapsed since his fungal thigh infection.

Conclusions: Aggressive surgical wound care with resolution of cutaneous zygomycosis enabled subsequent transplantation in this young infant with a favorable outcome.

9 High Serum B Cell Activating Factor Levels Inversely Correlate with low B Cells in both Common Variable Immune Deficiency and Good’s Syndrome

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Background: Causes for Common Variable Immune Deficiency (CVID) include: failure of B cell activation and maturation, altered somatic hypermutation and defective cell membrane signalling. CVID is associated with a reduction in class switched memory B cells. Similar impairments in B cell development have been thought to occur in Good’s Syndrome (GS). This disorder is characterized by thrombocytopenia, hypogammaglobulinemia, as well as a clinical phenotype of severe B and T cell immune deficiency. Early and systematic long term data are not available for most cases with GS and few cohorts have been studied systematically. B Cell Activating Factor (BAFF) and its receptor (BAFF-R) are necessary for B cell growth. BAFF is synthesized by cells of myeloid origin as a transmembrane protein and later cleaved to produce soluble BAFF. Stimulation of B cells with BAFF significantly retards their death and leads to B cell maturation. Low B cells are a poor prognostic indicator in CVID patients.

The role of BAFF as it relates to B cells in GS is unknown.

Objectives: We aimed to study the relationship of BAFF and BAFF-R to B cell memory phenotypes in CVID and GS.

Methods: We quantified BAFF and BAFF-R levels of 44 adults with CVID, 4 with GS and 25 healthy adult controls and compared the amount of BAFF and BAFF-R to the number of switched (CD27-IgD-) and unswitched (CD27-IgD+) B cells using anti-CD27, anti-CD19, anti-IgD, and anti-BAFF-R-FITC. Serum BAFF levels were measured using an enzyme-linked immunoassay.

Results: An absence of B cells occurred in all GS patients and significant decrease of class switched IgD-CD27+ B cells in CVID patients was noted when compared to controls (p<0.0001). There was no significant difference of switched IgD+CD27- levels between CVID patients to healthy donors (p>0.300). A significant difference between the serum BAFF of CVID and GS patients (p<0.01) as well as GS to controls (p<0.0002) was observed. Mean values were 12,678 pg/ml for GS and 3,070 and 968 pg/ml respectively for CVID and controls. Serum BAFF levels in CVID patients correlated with lower %B cells (p<0.0051) but no correlation was noted between BAFF levels of CVID patients to either IgD+CD27- (p>0.71) or IgD-CD27+ (p<0.58) B cells.

Conclusions: BAFF overexpression correlates with a lack of B cells in both CVID and GS. The significance of this association with respect to B cell development remains to be determined.

10 STAT3 Mutations in Job’s Syndrome

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Background: Job’s syndrome (HyperIgE) is an autosomal dominant multisystem illness manifested as pulmonary cysts and pneumonias, staphylococcal abscesses, eczema, elevated IgE levels, and bone and dental abnormalities caused by mutations of STAT3 gene (signal transducer and activator of transcription 3). STAT3 affects diverse targets including other inflammatory molecules.

Objectives: Describe the function of STAT3 DNA binding and SH2 domain mutations.

Methods: Six STAT3 mutation constructs within the DNA binding and SH2 domains were created by site-directed mutagenesis to re-