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 in regulated at many different steps. Definition of these pathways should allow for interventions to enhance anti-viral activity while decreasing inflammatory responses that are detrimental to the host.

### 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, hepatosplenomegaly, 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 of the wound (see figure) was allowed to heal by secondary intention following this he was transplanted with umbilical cord blood at the University of Minnesota.

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

![Figure 1. Deep and wide thigh wound after excision of zygomycotic lesion.](image)

**Figure 1.** Deep and wide thigh wound after excision of zygomycotic lesion.

### 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 unswitched 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-
flect Job’s mutations. Constructs were cotransfected into a STAT3 deficient cell line (Cos1) as well as a cell line with endogenous STAT3 (HeLa) along with a STAT3-responsive luciferase reporter vector. Peripheral blood mononuclear cells (PBMCs) from Job’s patients were used to measure STAT3 activation in nuclear extracts by STAT3 binding to DNA in an ELISA assay. NfκB nuclear translocation was detected with confocal microscopy using antibodies to p65 and p50. p38 MAP kinase activity was measured by ELISA. De-phosphorylation of STAT3 was examined by immunoblot.

Results: Mutations within both the DNA binding and SH2 domains result in a decrease of luciferase activity by 80-84% as compared with wild type after stimulation with oncostatin M. Cotransfection of wild type and mutant constructs together in Cos-1 cells resulted in a 24-63% loss of activity. IL10 plus lipopolysaccharide (LPS) stimulation of PBMCs from Job’s patients resulted in a 14-fold decrease in STAT3 activity as compared to normals as measured by ELISA. Following LPS stimulation, p38 MAP kinase phosphorylation between normal and Job’s cells were comparable, but NfκB activity was higher in Job’s cells. De-phosphorylation of STAT3 was normal in PBMCs from Job’s patients following treatment with staurosporine.

Conclusions: Job’s STAT3 mutations act in a dominant negative manner. Both DNA binding and SH2 mutations decrease activation of STAT3 in response to several stimuli impairing downstream signaling. These methods to evaluate the function of STAT3 mutants will help dissect mechanisms of STAT3 control of susceptibility to infection and control of inflammation.

11 The First Report of Somatic Mutation Reversion X-Linked Chronic Granulomatous Disease

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Introduction: X-linked chronic granulomatous disease (CGD) is caused by mutations in the CYBB gene, which impair gp91phox function and therefore lead to deficient superoxide generation by the phagocyte NADPH oxidase system. This leads to defective killing of certain bacteria and fungi and results in severe infections and granuloma formation.

Case: A 20-year-old man with X-linked CGD had a long history of classic CGD and severe inflammatory bowel disease requiring colonic diversion. He had two distinct populations of neutrophils and fungi and results in severe infections and granuloma formation.

Discussion: Somatic mosaicism due to site-specific reversions of inherited mutations to wild type has been described in other immunodeficiencies and has been linked to milder disease in some cases. This is the first report of a reversion mutation in X-linked CGD, and the only report of a reversion mutation affecting neutrophils in a primary phagocyte defect. Reversion mutations can occur in neutrophils, just as they do in lymphocytes and may be more common than previously thought.

12 Filamentous Fungal Pneumonias in Hyper IgE Syndrome

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Background: Autosomal dominant hyper-IgE syndrome (AD-HIES, Job’s syndrome) is a primary immunodeficiency characterized by recurrent staphylococcal abscesses, pneumonia, eczema, and elevated serum IgE. Connective tissue defects are also frequent. AD-HIES results from heterozygous hypomorphic mutations in the STAT3 gene, primarily affecting either the SH2 or DNA-binding domains. Filamentous fungal pneumonias (FFP), particularly with Aspergillus spp., have been reported in patients with clinically suspected AD-HIES. However, these reports pre-dated the ability to genotypically confirm the patients’ diagnoses and have been limited in sample size, precluding comprehensive understanding of this infection in AD-HIES. We sought to determine the prevalence of FFP in a large cohort of patients with confirmed AD-HIES.

Methods: Retrospective chart review of all AD-HIES patients followed at the NIH, for which the diagnosis was confirmed by genetic sequencing.

Results: 56 AD-HIES patients with STAT3 mutations were identified; 22 (39.3%) had no radiological evidence of lung cysts (includes cavities, pneumatocoeles), while 34 (60.7%) did. Among those without cysts, none (0%) developed FFP. Among those with lung cysts, 13 (38.2%) developed FFP. Aspergillus Neosartorya spp. were most common (11 total, 84.6%); infection with S. apiospermum (n=1) and H. capsulatum (n=1) also occurred. Multiple episodes of FFP frequently occurred in affected patients and did not occur exclusively in pre-existing cysts. Patients were stratified based on their mutation (SH2, n=28; DNA, n=28). Lung cysts were equally frequent in each group (n=17 for both). The number of patients in each group with lung cysts developing FFP was similar (SH2: 7/17 (41.2%); DNA: 6/17 (35.3%)) as was the median age at onset of 1st FFP (SH2: 30.5 yrs; DNA: 35.3 yrs). Treatment modalities varied and included antifungal agents (systemic, occasionally adjunctive intra-cavitary) with or without surgical resection. Mortality occurred more frequently in the DNA group (3/6; 50%) than in the SH2 group (0/7; 0%).

Conclusions: Patients with AD-HIES with lung cysts are at significantly increased risk for FFP, typically in their third decade of life. FFP do not necessarily occur only in pre-existing cysts/cavities, suggesting that cysts/cavities may be a marker for susceptibility rather than a cause of the infection. Aspergillus spp. are the primary pathogens. The mortality rate is significant and possibly related to genotype.

13 Herniated Nuclear Morphology and Cytoskeletal Anomalies in Neutrophils from Sisters with Recurrent Infections

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Background: Two Qatari sisters (5 and 12 yrs.) with a family history of consanguinity (parents are first cousins) and suspected neutrophil dysfunction had frequent severe skin and mucosal ulcer-