phosphorylation of STAT3 was examined by immunoblot. p65 and p50. p38 MAP kinase activity was measured by ELISA. De-
cation was detected with confocal microscopy using antibodies to
zyme. Following LPS stimulation, p38 MAP kinase 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 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 compar-
able, but Nfıb activity was higher in Job’s cells. Dephosphory-
lation of STAT3 was normal in PBMCs from Job’s patients following treatment with staurosporine.

Results: Mutations within both the DNA binding and SH2 domains result in loss-of-function but are dominantly negative. Mutant constructs in Cos-1 cells did not transactivate the STAT3 reporter and transfection in HeLa cells resulted 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 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 Nfıb activity was higher in Job’s cells. Dephosphorylation 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 sig-
aling. 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 in his peripheral blood and his buccal cells mutant, and only a small fraction of his peripheral blood neutrophils had the mutation 676C

Causing a premature stop (R226X) in SH2 group (0/7; 0%).

Discussion: Somatic mosaicism due to site-specific reversion 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 neu-

12 Filamentous Fungal Pneumonias in Hyper Ige Syndrome

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Background: Autosomal dominant hyper-IgE syndrome (AD-HIES) results from heterozygous hypomorphic mutations in the STAT3 gene, primarily affecting either the SH2 or DNA-binding do-
mains. 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 fol-
lowed 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, pneumatoceles), while 34 (60.7%) did. Among those without cysts, none (0%) developed FFP. Among those with lung cysts, 13 (38.2%) developed FFP. Aspergillus spp. Nosertorya 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 exclu-
sively 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 adjunc-
tive 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 signifi-
cantly 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 pri-
mary pathogens. The mortality rate is significant and possibly re-
lated 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 neutro-
thropil dysfunction had frequent severe skin and mucosal ulcera-
14 A Novel Autosomal-dominant Late-onset Immunodeficiency with Susceptibility to Mycobacteria, Fungi, Papillomavirus and Myeloid Malignancies

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Background: Primary immunodeficiencies characterized by selective predisposition to non-tuberculous mycobacteria (NTM) disease include autosomal dominant and recessive disorders of the IL-12/IL-23/IFN-γ axis including STAT1, and X-linked recessive defects in NFκB-essential modulator (NEMO). In the process of evaluating patients with disseminated NTM infections, we encountered a discrete group of patients whose clinical course, laboratory features, and family histories indicate a novel immunodeficiency.

Materials/Methods: We identified patients with severe NTM infection, for whom mutations in the genes of the IL-12/IL-23/IFN-γ axis including STAT1, and X-linked recessive defects in NFκB-essential modulator (NEMO). In the process of evaluating patients with disseminated NTM infections, we encountered a discrete group of patients whose clinical course, laboratory features, and family histories indicate a novel immunodeficiency.

Materials/Methods: We identified patients with severe NTM infection, for whom mutations in the genes of the IL-12/IL-23/IFN-γ axis including STAT1, and X-linked recessive defects in NFκB-essential modulator (NEMO). In the process of evaluating patients with disseminated NTM infections, we encountered a discrete group of patients whose clinical course, laboratory features, and family histories indicate a novel immunodeficiency.

Results: Thirty-nine patients (17 men, 22 women), 25 to 85 years old with ICL were evaluated and thirty-six were followed for a median of 49.5 months (range). Most of the patients presented with cryptocoecal and non-tuberculous mycobacterial infections, while seven patients presented with no infection (incidental finding of low CD4 T cell count). In thirty-two patients, CD4 T cell counts remained below 300 cells/mm³ during the entire study period without progression of lymphopenia and in seven normalized after an average of 31 months. Using a linear mixed model approach to fit the individual CD4 trajectories, the CD4 velocity was estimated at 0.66/mm³/month (95% CI: -0.20, 1.54) and was not significantly different from zero (p=0.13). Overall, fifteen patients (41.6%) developed an opportunistic infection in follow up (mostly HPV, VZV infections and candidiasis), five (13.8%) of which were AIDS defining clinical conditions, and four patients (11.1%) developed autoimmune diseases. Patients without opportunistic infections (OIs) at presentation remained asymptomatic. Seven patients died, four from ICL related opportunistic infections within 42 months of diagnosis. CD8 T lymphocytopenia (<180/mm³) and high CD4 T cell activation (measured by HLA-DR expression) at presentation were associated with adverse outcome (ICL related death) (p=0.003 and 0.02 respectively).

Conclusions: ICL is a heterogeneous yet distinctive, clinically and immunologically, from HIV infection condition. CD4 T cell counts in ICL remain stable or improve in the majority of patients but life-threatening OIs and autoimmune diseases can occur.