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. Dephosphorylation of STAT3 was examined by immunoblot.
Results: Mutations within both the DNA binding and SH2 domains result in loss-of-function but are dominant 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 NFκB 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 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.

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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 by neutrophil oxidative burst testing of peripheral blood. In two independent measurements of neutrophil oxidative burst by dihydrorhodamine (DHR) assay, 97% of peripheral blood neutrophils failed to oxidize dihydrorhodamine (DHR-), while 3% had a normal oxidative capacity (DHR+). Sorted DHR+ and DHR- cells were studied by microsatellite analyses, which proved patient origin of both the DHR+ and DHR- cells. The DHR- cells and buccal mucosal epithelial cells and unsorted peripheral blood mononuclear cells had the mutation 676C>T, which causes a premature stop (R226X) in CYBB. In contrast, the DHR+ cells had the wild-type sequence, 676C leading to a normal protein. Therefore, since he had the majority of his peripheral blood and his buccal cells mutant, and only a small percentage reverted to normal, we believe that he has reverted the mutation in a small population of his neutrophil precursors. Exploration of other cell lines (lymphoid, monocyte, etc) is underway.
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.

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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, 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/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: 35.5 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.

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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 ulcera-