Supplementary material

Case 1: II.2 (C244T/C244T)

A 9 year-old female born to a first cousin consanguineous union had an otherwise unremarkable medical and developmental history other than having received a routine course of isoniazid treatment at age 3 for having had contact with an active case of tuberculosis. At age 9, she presented with intermittent fever treated with oral antibiotics for suspected bacterial infections. One week later, she presented with 2 days of fever, ataxic gait and slurred speech. She had upward and lateral gaze nystagmus, generalized hypotonia, bilateral tremors, and head titubation with intact cranial nerves. She required intensive care and mechanically assisted ventilation. Peripheral hematological counts were: normal Hemoglobin (Hb), white blood cell count (WBC) of 3,700 cells/µL (absolute neutrophil count (ANC) 1,665 cells/µL, and absolute lymphocyte count (ALC) 1,998 cells/µL) and 350,000/µL platelets. Except for elevated protein (600 mg/L), cerebrospinal fluid (CSF) was normal and negative for bacteria, mycobacteria, and fungi. Serum and CSF were negative for cytomegalovirus (CMV), herpes simplex virus, enteroviruses and varicella virus by PCR. Head CT scans were normal and she was treated with one dose of IVIG for suspected Guillain-Barré syndrome and 10 days of intravenous ceftriaxone, azithromycin and acyclovir. Fever persisted (40°C) and she was diagnosed with pneumonia. One week later, she developed worsening ataxia and speech with profound right-sided hypotonia, loss of consciousness, loss of gag and abdominal reflexes, dilated but reactive pupils and she required assisted ventilation. Hb was 10.6 g/dL, WBC of 1,650 cells/µL (ANC 501 cells/µL, ALC 890 cells/µL), and platelets of 131,000/µL. Serum ferritin and triglycerides were normal. Head magnetic resonance imaging (MRI) showed meningoencephalitis with predominant posterior fossa involvement. Bone marrow aspirate revealed hypocellularity and only evidence of reactivity without other abnormality. Her immunoglobulin levels were normal, with the exception of elevated IgE (440 Ku/L). Analysis of CSF demonstrated elevated protein (1.08 g/L) without cells and no bacterial growth. Lymphocyte subsets analysis was remarkable for lymphopenia (ALC 880 cells/µL),

with 95% $CD3^+$ T cells (863 cells/μL), 52% $CD4^+$ T cells (458 cells/μL), 40% $CD8^+$ T cells (352 cells/μL), 2% $CD19^+$ B cells (176 cells/μL), and 0.7% $CD56^+$ NK cells (62 cells/μL). She developed pulmonary hemorrhage and ganciclovir treatment was started providing owing to CMV having been identified in her bronchoalveolar lavage (BAL) specimen.

Four months later, head MRI was repeated and demonstrated generalized large lesions in right frontal lobe as well as extensive low attenuation changes within the midbrain. An open lung biopsy was performed and histological examination identified only lymphocytic interstitial pneumonia. She was presumed to have and was treated for hemophagocytic lymphohistiocytosis (HLH) with high-dose of dexamethasone, anti-thymocyte globulin (ATG), mycophenolate mofetil (MMF) and alemtuzumab (anti-CD25 mAb). She was weaned from ventilator support but she had bulbar palsy, left-sided hemiplegia, and ataxia and required a tracheotomy with ongoing mechanical ventilatory assistance. Several months thereafter she was diagnosed with septic shock and died due to acute respiratory distress syndrome. Sanger sequencing for *RAB27A* detected that the patient was homozygous for c.244C>T (p.R82C) which retrospectively confirmed the diagnosis of HLH.

Case 2: II.1 (?/?)

A 10 year-old male was previously generally healthy and like his sister (Case 1) was treated with INH prophylaxis for an exposure to active tuberculosis. He had the additional medical history at age 6 of needing treatment for brucellosis (*B. melitensis* titer 1:5,120) with 3 weeks of rifampicin and trimethoprim-sulfamethoxazole (TMP-SMZ). At age 10, he developed fever with hemoglobin (Hb) of 8.9 g/dL, WBC was 2,400 cells/μL (ANC of 800 cells/μL, ALC of 1,300 cells/μL), platelets of 121,000/μL and serum ferritin of 81 μg/L. One week later, he developed hepatosplenomegaly, with normal liver function test (LFTs). After three months, he developed mild jaundice with a serum unconjugated bilirubin of 60 μmol/L, and conjugated bilirubin <2.0. Serum PCR was negative for adenovirus, CMV, and Epstein-Barr Virus (EBV). Serum IgG was 1,200 mg/dL, IgA at 147mg/dL, and IgM of 107 mg/dL. He was treated with oral prednisolone, cyclosporine, TMP-SMZ prophylaxis and IVIG. The hepatosplenomegaly improved thereafter with normalizing cell counts (except for platelets of 121,000/μL). At 11 years of age he developed headache, diplopia, and muscle weakness with preference for lower extremities. Head MRI demonstrated hemorrhagic lesions in the frontal and non-hemorrhagic lesions in the right mesial lobes. Serum virological studies detected EBV DNA (1,779 copies/ml). CSF cultures including those for acid-fast bacilli as well as PCR for enteroviruses, John Cunningham virus (JCV), BK virus and parvovirus B19 were all negative. His skin biopsy and that of his sister both were the same, revealing monoclonal T-cell populations (mixed CD4 and CD8 cells). Over 4 weeks, he deteriorated with progressive pancytopenia, multiple intracranial hemorrhagic lesions and intractable seizures, demyelinating sensorimotor neuropathy, and other infections. He did not respond to intensive care-level treatment including mechanically-assisted ventilation, IV antimicrobials, daclizumab (anti-CD25 mAB), alemtuzumab, high-dose dexamethasone, and he subsequently died.

Case 3: II.3 (C244T/wt)

The patient is male and has been evaluated for familial HLH syndromes since childhood owing to his family history. He had an unremarkable course. Repeated CBC, WBC with differential counts, platelet counts, liver function tests and kidney function tests were normal. Serum IgG, IgA, IgM, IgG subclasses, anti-vaccine antibody titers, and lymphocyte subsets were normal except for low CD56. PCR for CMV, EBV, and adenovirus were negative. Between 11 – 16 years of age, he had low to borderline-normal NK cell numbers (4-5% of lymphocytes) and decreased to absent NK cell function (average 0.1 L.U.; n=7). CD107a mobilization was normal (15%; MCF 165 (range 10-37%; MCF 152-986)).

Case 4: II. 4 (C244T/C244T)

The patient is male and has been evaluated for familial HLH syndromes since the age of 8 owing to his family history. He had an unremarkable course except for persistence of low EBV viremia (64-562 copies/μg) for 5 years. PCR for CMV and adenovirus were negative. Between 7 – 12 years of age, his NK cell numbers were normal (7-15% of lymphocytes), with decreased to absent NK cell function (average L.U. <0.3; n=7). CD107a mobilization upon activation was low (10%; MCF114 (range 10-37%; MCF 152-986)).

Case 5: II.5 (C244T/C244T)

The patient is male and has been evaluated for familial HLH syndromes since the age of 5 owing to his family history. He had an unremarkable course. Between 6 and 10 years of age, he had EBV viremia (80-932 copies/µg), but PCR for CMV and adenovirus were negative. CMV serology was positive documenting past infection. His lymphocyte subsets were essentially normal. Between 5 – 10 years of age, his NK cell numbers were 6-17% of lymphocytes, but NK cell function was decreased to absent (average L.U. 0.09; n=5). CD107a mobilization upon activation was decreased (6%; MCF 75 (range 10-37%; MCF 152-986)).

Case 6: II.6 (C244T/wt)

The patient is female and has been evaluated for familial HLH syndromes since the age of 5 owing to his family history. She had an unremarkable course. Repeated CBC, WBC with differential counts, platelet counts, liver function tests and kidney function tests were normal. Serum IgG, IgA, IgM, IgG subclasses, anti-vaccine antibody titers, and lymphocyte subsets were normal. Between 2 and 5 years of age, she had low EBV viremia (80-219 copies/μg), which has subsequently returned to undetectable levels. Quantitative studies for CMV and adenovirus were negative. Between 1 – 5 years of age, her NK cell numbers were normal (5-11% of lymphocytes) and NK cell function was absent to borderline-normal (average 0.9 L.U.; n=3). CD107a mobilization upon activation was normal (14%; MCF 206 (range 10-37%; MCF 152-986)).

Case 7: II.7 (C244T/wt):

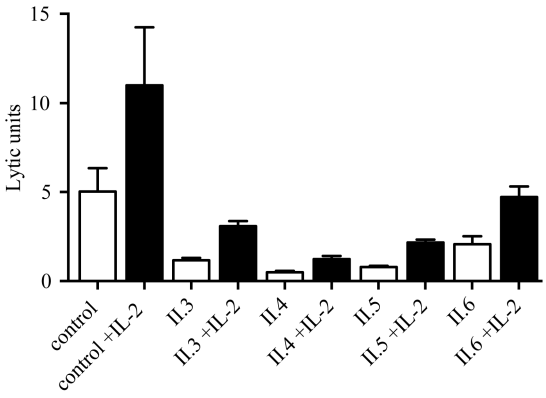
The patient is male and has been evaluated for familial HLH syndromes since birth owing to his family history. He had an unremarkable course. Immunological studies were normal, as were quantitative tests for adenovirus, CMV, and EBV in the peripheral blood. At 2 years of age, NK cell frequency was 13% of lymphocytes, but NK cell function was decreased to absent (average 1.0 L.U.; n=2) with decreased CD107a mobilization upon activation (9%; MCF 149 (range 11-35%; MCF 207-678).

Supplemental figure legend

Figure E1: NK cell mediated cytotoxicity is partially rescued by interleukin (IL)-2. Lytic units as number of NK cells x10^7 required to mediate 20% of the target cell lysis were used as a measurement of the cytolytic capacity and were determined as described preciously (1). PBMCs from a healthy control and the patients were either untreated (white bars) or incubated with IL-2 30 min prior to the standard 4h ^{51}chromium release assay against K562 target cells (black bars) (n=2).

Supplemental References

1. Orange JS, Chehimi J, Ghavimi D, Campbell D, Sullivan KE. Decreased natural killer (NK) cell function in chronic NK cell lymphocytosis associated with decreased surface expression of CD11b. Clinical Immunology. 2001;99(1):53-64.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