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BRIEF REPORT

Late relapse of primary hemophagocytic lymphohistiocytosis after hematopoietic stem cell transplantation: a consequence of low-level chimerism from a carrier donor?

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To the editor,

Familial hemophagocytic lymphohistiocytosis (FHLH) is a genetic, rapidly progressive, life-threatening immune disorder characterized by uncontrolled, exaggerated systemic inflammation. Several autosomal recessive defects related to function of the perforin/granzyme-dependent cytotoxic pathway are known [1]. Mutations in genes encoding perforin, Munc 13-4, Munc 18-2, and syntaxin-11 are identified as causes of FHLH, but cases without a known genetic defect may be diagnosed based on positive family history, parental consanguinity, and refractory or recurrent HLH suggestive of a genetic predisposition [1]. Deficient cytotoxic activity of NK and T-lymphocytes is responsible for the pathological features of the disease. Failure of antigenic clearance and cytotoxicity-mediated homeostatic deletion of cytotoxic T-lymphocytes and antigen-presenting cells instigates accumulation of activated polyclonal CD8 T-lymphocytes and activated macrophages within various organs, including the central nervous system, causing a hypercytokinemic hyperinflammatory state [1, 2].

In specific situations, disease relapse can rarely follow hematopoietic stem cell transplantation (HSCT) for FHLH, usually soon after transplantation [3]. Mixed donor chimerism is tolerable, with a minimum of 20–30% donor alleles in the CD3+ compartment typically sufficient to control the disease [3]. We report an infant who required two HSCT for FHLH, specifically highlighting questions of how much donor chimerism is adequate for HSCT to be curative and whether heterozygous carrier status of the donor affects transplant outcome.

The second child of a highly consanguineous family was born at term and received BCG vaccination soon after birth. At 2 months of age, after receiving the first routine immunizations, she developed fever and drowsiness with cervical lymphadenopathy, hepatosplenomegaly, neutropenia, thrombocytopenia, and hypofibrinogenemia, which did not respond to broad-spectrum antibiotics. Ferritin and triglycerides were also raised. Microbiological investigations were negative. Cerebrospinal fluid was normal and thoracic computerized tomography only showed bilateral pleural effusions. Bone marrow aspirate and lymph node biopsy demonstrated hemophagocytosis (Fig. 1a–c). Suspecting likely FHLH, the HLH-94 protocol was started with dexamethasone (10 mg/m^2) and etoposide (150 mg/m^2, 3 doses). After a good initial response, with improvement of organomegaly, blood counts, and liver function, two life-threatening episodes of HLH, with recurrence of the initial findings and severe respiratory distress, required treatment with cyclosporine, methylprednisolone (maximum dose 5 mg/kg/day), and anti-thymocyte globulin (ATG, 10 mg/kg, 5 doses).

Because of the severe clinical presentation and partial response to treatment, she was transplanted from her healthy

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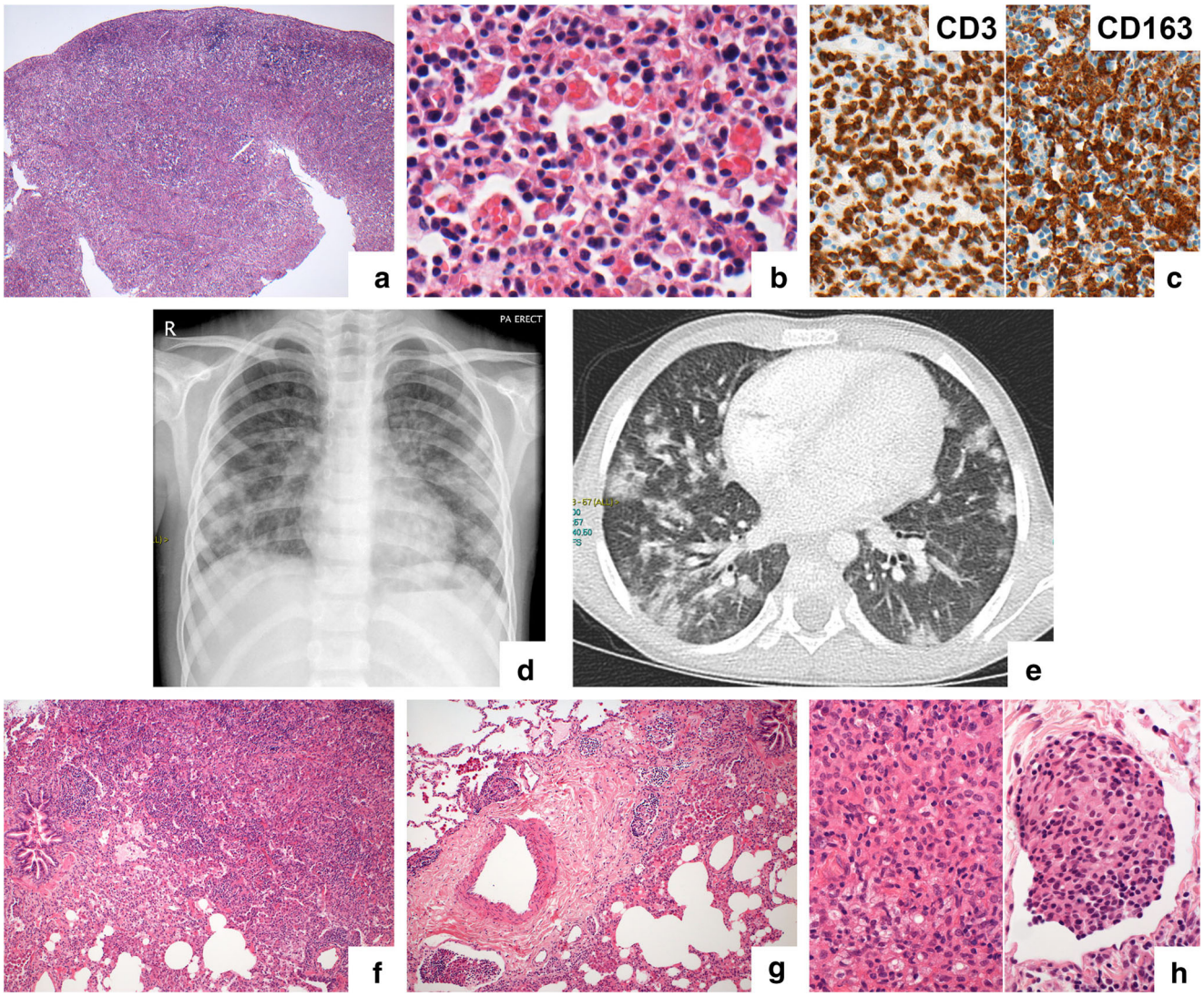

Fig. 1 Histopathology and radiology. **a**–**c** Lymph node histology at diagnosis showing architectural effacement (**a**) by a lymphohistiocytic infiltrate with prominent erythrophagocytosis (**b**). Numerous CD3-positive T cells and CD163-positive macrophages were present (**c**). **d**, **e** Lung imaging at relapse: CXR demonstrates widespread pulmonary infiltrates and opacities without pleural effusions. CT confirms diffuse centrilobular nodularity with perinodular ground-glass change, features consistent with an inflammatory process. **f**, **g** Lung histology at relapse showing patchy but marked lymphohistiocytic infiltration of alveolar walls and airspaces forming areas of consolidation with a predominantly centrilobular distribution (**f**). Cohesive aggregates of lymphoid and histiocytic cells filled expanded lymphatic vessels around bronchovascular bundles and along intralobular septa (**g**). The macrophage-rich infiltrate is shown at high power (**h**) in parenchyma (left panel) and a lymphatic space (right panel)

HLA-identical sister. Conditioning was performed using the then current EBMT Inborn Errors Working Party protocol for HLH, with ATG (5 mg/kg/day, 5 doses), busulfan (4 mg/kg/day, 4 doses), and cyclophosphamide (50 mg/kg/day, 4 doses). Cyclosporine and methotrexate were used as graft-versus-host disease (GvHD) prophylaxis. She demonstrated good immunoreconstitution, with neutrophil and platelet engraftment on day + 23 and day + 32 post-HSCT respectively. Whole blood chimerism studies on day + 40 showed predominant donor, with a trace of recipient alleles, but by + 100 days she had a mixture of donor and recipient alleles.

At 10 months post-HSCT, lineage-specific chimerism assays demonstrated donor and recipient alleles, with a higher level of recipient alleles in all cell fractions. Immunological investigations demonstrated normal numbers of naïve T lymphocytes and class-switched memory B lymphocytes, normal lymphocyte proliferation, and normal immunoglobulin levels, with antibody responses to vaccine antigens. She had no severe infections. The first quantitative chimerism testing was available 2.5 years after transplantation and revealed 30% donor alleles in T and B lymphocytes and 15% in myeloid cells, which remained stable.

Six years post-HSCT, she developed fever, neutropenia, and thrombocytopenia. Bone marrow aspiration revealed hyper-cellularity with occasionally activated histiocytes but no lymphohistiocytic infiltrate or hemophagocytosis. She

recovered spontaneously within a week and the episode was diagnosed as transient virus-induced cytopenia. During the next year, she presented recurrent similar episodes of fever, lasting 4–5 days, every 5–7 weeks, with no obvious source, with occasional abdominal pain, headache, arthralgia, and non-specific rash. When perforin mutations were identified as a cause of FHLH, genetic testing was performed and a homozygous *PRF1* mutation was identified (1284G>A, Trp428*), predicted to stop protein translation. Her sister was identified as a heterozygous carrier. No functional testing was performed.

Seven years post-HSCT, she presented with fever, neutropenia, thrombocytopenia, hypofibrinogenemia, hypertriglyceridemia, hepatosplenomegaly, respiratory distress, and lung infiltrates, demonstrated on chest radiographs and computerized tomography (Fig. 1d, e). Hemophagocytosis was absent in the bone marrow but a lung biopsy showed florid lymphohistiocytic inflammation (Fig. 1f–h), confirming relapsed HLH with reduced donor chimerism. Remission was induced with the HLH-2004 protocol and she was retransplanted using the same sibling donor, due to lack of a matched unrelated donor, with fludarabine (30 mg/m^2, 5 doses) and melphalan (140 mg/m^2) conditioning and mycophenolate mofetil and cyclosporine as GvHD prophylaxis. With a 10-year follow-up after the second procedure, she remains clinically normal on no medication, with 100% donor chimerism in all cell lineages and normal immune reconstitution.

Relapsed HLH after HSCT is reported, mostly related to primary graft failure (more frequent in active disease and haploidentical HSCT) or secondary graft loss (observed more in haploidentical and partially mismatched donors and reduced intensity conditioning) [3, 4]. Most HLH relapse is observed early after HSCT, but the latest reported cases as late as 6.7 years, preceded by good initial donor engraftment but then development of mixed chimerism and a slow decline of donor contribution to hematopoiesis/lymphopoiesis [3]. Our case is also a late FHLH recurrence, 7 years after successful HSCT. Mixed chimerism is frequent after HSCT in FHLH, since introduction of reduced intensity conditioning regimens [3]. Donor incompatibility is a risk factor for mixed chimerism, as is active disease at the time of the transplantation [2, 4], the latter probably due to persistent hypercytokinemia and/or marrow infiltration by activated T lymphocytes and macrophages, which inhibit hematopoiesis and impair donor stem cell engraftment (Table 1).

Usually, 20% donor chimerism is sufficient to maintain stable complete remission [3], possibly because the remaining donor lymphocytes exert a trans-regulatory effect on recipient T lymphocyte expansion and macrophage activation triggered by an infection. We propose that the combination of low donor chimerism and the heterozygous perforin mutation of donor cells contributed to disease relapse in our case. The evolutionarily conserved Trp428 residue mutated in the family we report is located within the calcium binding region 1 (CBR1) at the N-terminal end of the C2 domain of perforin, which is critical for membrane binding and cytolytic activity [5]. Notwithstanding any dominant negative effect of the mutant protein, it is possible that the heterozygous Trp428* mutation resulted in a reduced pool of functional perforin protein in donor cytotoxic lymphocytes, leading to impaired immune control. Although the clinical significance of heterozygous *PRF1* variants in the general population is controversial, heterozygosity for some variants, such as the hypomorphic Ala91Val allele, is associated with reduced NK cell cytotoxicity in vitro or has been implicated in HLH or macrophage activation syndrome in other immunodeficiency or autoinflammatory contexts [6–8]. Mice heterozygous for a null perforin allele (*Prf1*±) displayed an approximately 50% reduction in cytotoxicity compared to wild-type littermates [6]. Patients transplanted from carrier donors have been reported [4], but there are no clear data on the donor chimerism or whether this led to disease relapse. Our experience suggests that donor T lymphocyte chimerism should be carefully monitored, with higher percentages targeted, in such situations, with vigilance for atypical manifestations of HLH relapse. Importantly, level of perforin expression will be critical achieving cure in patients treated with gene therapy [9].

Table 1 Evolution of donor chimerism over time for transplant 1 and transplant 2

Time post HSCT	WB chimerism	Myeloid	B cell	T cell
HSCT 1				
D + 46	Mixed			
D + 103	Mixed			
D + 285	Mixed M > T + B			
D + 355	Mixed M > T + B			
2.67 years		15%	30%	30%
3.58 years		15%	25%	36%
6.25 years		24%	23%	33%
6.58 years		22%	–	29%
HSCT 2				
D + 25	100%			
D + 45	100%			
D + 115	100%			
1 year	100%			
2.5 years	100%			
3.5 years		100%	100%	100%
4.5 years		100%	100%	100%
6 years	100%			
7 years	100%			
9 years		100%	100%	100%

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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