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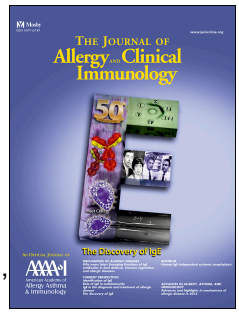

Bone marrow transplantation for MHC class I deficiency corrects T cell immunity but dissociates NK cell repertoire formation from function

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Bone marrow transplantation for MHC class I deficiency corrects T cell immunity but dissociates NK cell repertoire formation from function.

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Capsule Summary

We describe the first bone marrow transplant undertaken for MHC class I deficiency, highlighting the improvement of both clinical and immunological function in this MHC class I chimeric setting.

Clinical Implication

Human MHC class I deficiency can be successfully transplanted following full reconstitution of the CD8 compartment and partial NK reconstitution with chimeric tolerance of MHC class I low somatic tissue.

To the Editor

The examination of primary immunodeficiency (PID) represents an opportunity to gain unique insights into models of human immunity[1]. MHC class I-deficiency is a PID where the both innate and adaptive immune systems are compromised due to the effects of absent MHC class I upon $CD8^+$ T-cell and NK-cell development and function[2]. The most frequent cause of MHC class I-deficiency is loss of the TAP1 or TAP2 proteins. These proteins import peptides from the cytoplasm to MHC class I molecules within the endoplasmic reticulum, supporting their peptide-loading and cell surface expression. $CD8^+$ T-cells require MHC class I expression for their thymic selection and development. Mature $CD8^+$ T-cells target infected tissues by recognizing foreign peptides presented by MHC class I molecules on the surface of infected-cells. NK-cells also require MHC class I engagement for full functional development during their ontogeny[3]. However, once fully functional, NK-cell effector functions are inhibited by MHC class I [4].

We describe the successful outcome and normalization of T-cell immunity after the first allogeneic stem-cell transplant for MHC class I-deficiency. The child, born in Pakistan to first cousin parents, presented at the age of six years old with a two year history of recurrent *Streptococcus pneumoniae* and *Haemophilus influenzae* chest infections, with radiological evidence of bronchiectasis. From the age of ten years she developed chronic, slowly enlarging skin ulcers over the extensor surfaces of both elbows (Figure 1a). HHV and EBV were identified from skin biopsies by polymerase-chain reaction but blood remained negative for these and other viral type including CMV (data not shown). The ulcers failed to respond to antibiotics and anti-viral agents. A male second cousin, had a similar

clinical phenotype with bronchiectasis and chronic skin ulcers, died at the age of ten years of cor-pulmonale secondary to severe chronic lung disease.

Initial immune investigations revealed a distinctive phenotype of a high CD4/CD8 ratio (10:1) with low numbers of $CD8^+$ T-cells ($0.29x10^9$/L) and normal immunoglobulins (Table I). Due to the child's poor clinical response to conventional treatment, continued deterioration in respiratory function, skin ulcers, abnormal immune function and family history, she underwent a stem-cell transplantation for an undefined-immunodeficiency. She was homozygous across the HLA (A2402/-, B3502/-, Cw0401/-, DRB10301/-, DQB10201/-, DPB10501/-) and transplanted with a partially matched unrelated female donor (A2407/-, B3503/-, Cw0401/-, DRB10301/-, DQB10201/-, DPB10501/-) at the age of 13 years old. This followed low intensity conditioning with campath 1H 0.2mg/kg dose (x4), fludarabine 30mg/m^2/dose (x5) and melphalan 140mg/m^2/dose (x1). Her clinical course post-transplantation was uncomplicated. At six months post-transplant the patient was 100% donor chimeric in both the myeloid and lymphoid lineages. Her clinical condition had improved and the large ulcers had healed (Figure 1b).

In view of her clinical presentation, immunological abnormalities and homozygosity at the MHC, she was extensively investigated for MHC class I-deficiency.

Total surface MHC class I expression(A2402, B3502, Cw0401) on the patient's pre-transplant PBMC showed a reduction in surface MHC class I expression (Figure 1c). This expression was notably higher than previous reports from patients with MHC class I-deficiencies[5]. Flow cytometry using locus specific

antibodies for the TAP dependent HLA-C and E alleles demonstrated an absence of both alleles (Figure 1c). These two alleles are key ligands for NK-cell ontogeny and function. The patients Cw0401 allele is cognate for the KIR2DL1 receptor whilst HLA-E is recognized by NKG2A, these are the only cognate MHC-KIR interactions present in this individual. Evaluation of patient's PBMC utilizing intracellular flow cytometry for TAP1 and TAP2 proteins, demonstrated absence of both proteins pre-transplant (Figure 1d). Molecular studies demonstrated truncation of TAP1 cDNA after exon 3 and complete absence of TAP2 cDNA. Genomic DNA evaluation identified a homozygous deletion from exon 3 of TAP1 through to exon 11 of TAP2, confirming the first description of a combined TAP1 and TAP2 deficiency (Supplementary Figure 1).

Post-transplant the MHC class I expression returned to normal (Figure 1b) with restoration of TAP1 and TAP2 expression by flow cytometry (Figure 1d). At six months post-transplant the $CD8^+$ T-cell population increased to 45% of T-cells, and the CD4:8 ratio corrected to 1:1 (Table I). We evaluated the TCR Vβ profile post-transplant to establish the reconstitution of a polyclonal repertoire of T cells. The TCR profile of the post-transplant $CD8^+$ T-cells at 1 year showed broad representation of all T-cell receptor Vβ family members (Figure 2a). Additional naïve T-cell phenotyping showed that 53% of $CD4^+$ and 11% of $CD8^+$ T-cells were naïve. T-cell proliferation studies returned to normal and all parameters were stable over the first 36 months post-transplant (Table I).

The theoretical concern post-stem-cell transplantation for MHC class I-deficiency is that donor NK-cells will be successfully reconstituted following cognate

engagement with MHC class I-competent hematopoietic tissue and this may lead to deleterious self-reactivity with MHC class I-deficient tissue. Post-stem-cell transplantation, the NK-cell distribution returned to normal representing 22% of lymphocytes with 7% of them being $CD56^{bright}$ compared to 46% pre-transplant (Table1). NK-cells also displayed a different phenotype post-transplant whereby they lost the unusual phenotype of being multi KIR positive dropping from 43% pre-transplant to <2% of NK-cells (Table 1). In addition the post-transplant NK-cells displayed a reconstitution of the expected single positive cognate KIR2DL1:HLA-Cw4 population (Table 1). Despite these changes in NK cell phenotype, the functional deficiency of the NK-cells remained impaired post transplant, showing no difference to pre-transplant NK-cells in cytokine and degranulation assays against MHC class I deficient target cells (Figure 2b).

In MHC class I deficiency, the NK-cell population have not encountered cognate MHC class I ligands and fail to proceed to full functional development[6-8]. Similarly, CD8 T cell have failed to be selected by MHC class I complexes within the thymus leading to poor development. In the post-transplant setting, hematopoietic reconstitution has supported CD8 T cell development, leading to naïve and TCR diverse populations. For NK-cells, there is evidence of engagement with the restored MHC class I-competence of the hematopoietic lineage leading to phenotypic change but this does lead to full functional restoration. This NK cell hypofunction may protect against self-reactivity to MHC class I-deficient somatic tissue. In summary this patient has improved dramatically following BMT for MHC class I deficiency and now exhibits a

distinctive immune status with impaired NK function but restored CD8 immunity that will require long term follow up.

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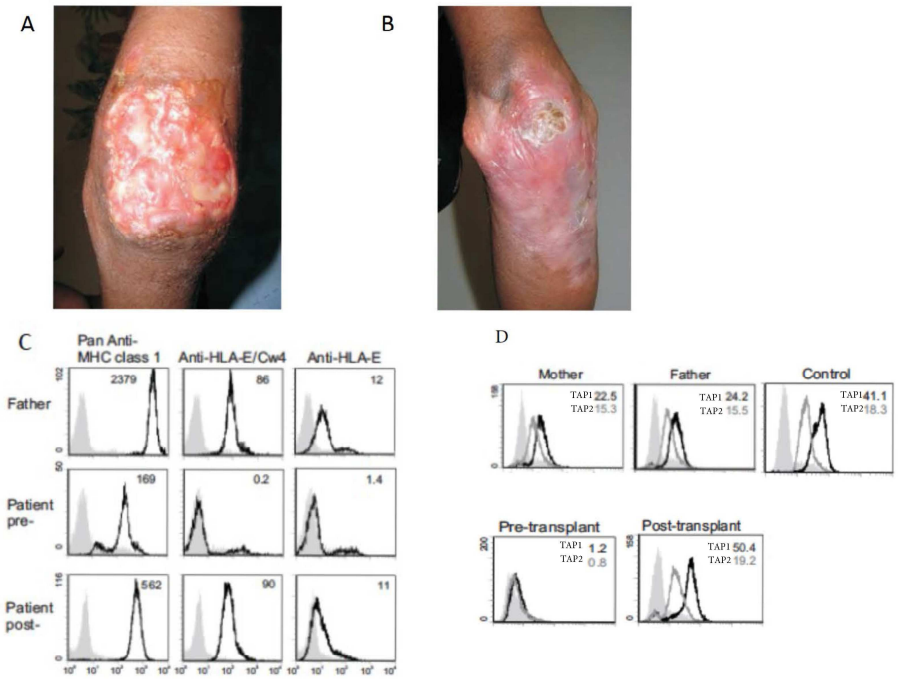
Figure Legends

Figure 1 Clinical Presentation of the patient showing (A) Pre-transplant necrotic ulcer of the right elbow, and (B) Post-transplant healed ulcer of the right elbow at 12 months. (C) Flow cytometry of MHC class I expression on PBMC taken pre- and post-transplantation, compared to that of the father (HLA-A2/24; HLA-B35/61; HLA-Cw4/Cw15). Isotype control (filled histograms) and specific anti-HLA antibodies (black unfilled histograms) are shown together with the MFI of MHC class I expression. (D)TAP expression by intracellular flow cytometry of patient BLCL pre and posttransplantation compared to that of the father, mother and healthy control. Cells were stained for TAP1 (TAP1.28, unfilled histograms black line), TAP2 (TAP2.17, unfilled histograms grey line) or relevant isotype control (filled histograms). Histograms were gated on live lymphocytes. The MFI of the TAP1 and TAP2 expression is shown.

Figure 2 (A)Flow cytometric analysis of T cell receptor Vβ family expression on pre- transplant and 12 month post-transplant T cells (left panel) and on the CD4 or CD8 T cell subsets post-transplant (right panel). The frequencies of the individual Vβ family within each subpopulation are shown as a percentage of that particular subset. (B) Comparison of NK cell function pre- and posttransplant (18 months) with three healthy controls. Shown are the percentages of CD3-veCD56+veNK cells expressing either IFNγ by intracytoplasmic cytokine staining or degranulating (CD107a expression) in response to IL-15, K562 or IL-15 and K562, as determined by flow cytometry.

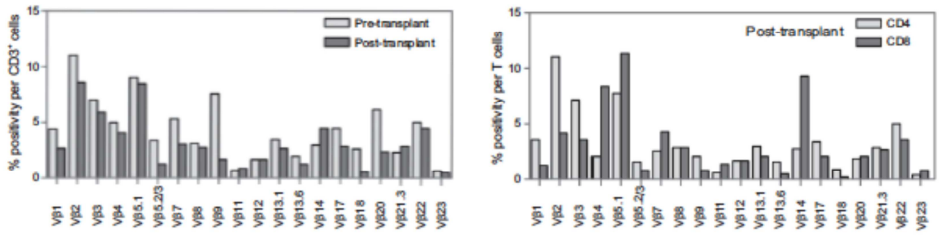
Figure 1.



12 months. (C) Flow cytometry of

Figure 2.

A



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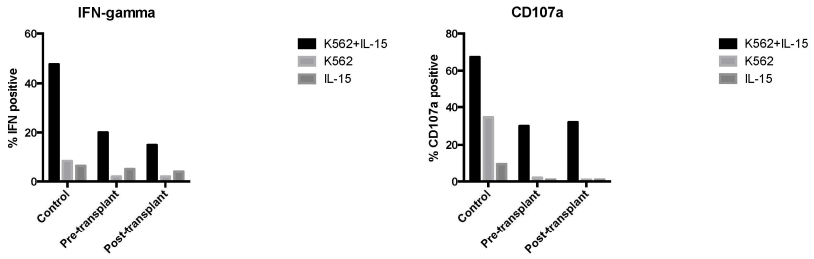


Supplementary Table 1 Lymphocyte phenotype and *in vitro* proliferative responses at pre- and post-transplantation time-points.

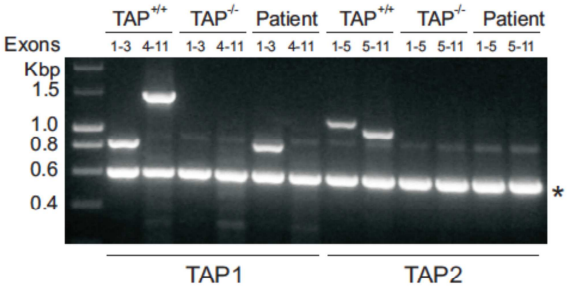
	Pre-transplant	Post-transplant	Reference range
Lymphocytes ($x10^9$/L)	4.2	3.7	1-5
T cell - Enumeration			
CD4 ($x10^9$/L)	3.0	1.2	0.4-2.1
CD8 ($x10^9$/L)	0.29	0.9	0.2-1.2
CD4/CD8 ratio	10	1.3	1-3.6
Naïve CD4 (% of $CD4^+$ T cells)	72	53	25-55
Naïve CD8 (% of $CD8^+$ T cells)	88	11	10-50
TCR Vβ expression	82	86	>70%
T cell - Function			
unstimulated (cpm)	261[1]	288[2]	1,435[1] / 376[2]
anti-CD3 (cpm)	5,638[1]	136.617[2]	158,306[1]/ 201,034[2]
anti-CD3 + interleukin-2 (cpm)	477,291[1]	261,708[2]	464,012[1]/ 280,034[2]
PMA (cpm)	7,003[1]	125,751[2]	299,981[1]/ 114,851[2]
PMA + ionophore (cpm)	380,870[1]	177,818[2]	291,281[1]/ 182,116[2]
PHA (cpm)	146,845[1]	270,906[2]	259,052[1]/ 271,222[2]
ConA (cpm)	316,114[1]	227,533[2]	256,313[1]/ 178,131[2]
NK cell - Enumeration (% NK-cells)			
NK cells ($x10^9$/L)	0.13	0.8	0.07-1.2
$CD56^{bright}$ NK cells (% NK-cells)	46	7	1-10

Triple KIR/NKG2A^{+} NK-cells	43	<2	<2
Cognate MHC-KIR^{+} NK-cells	<2	37	ND

Supplementary Table 1
cpm- counts per minute ND -not determined
1Control value for pre 2 Control value for post
Naive T cells were identified as CD45RA CCR7+ve
Triple KIR/NKG2A NK cells are represted by those that co-express KIR 2DL1, 2DL2/3 and KIR 3DL1/NKG2A Cognate MHC-KIR+ NK cells are presented by those co-express KIR 2DL1 (cognate for cw0401)

Supplementary Figure 1

A



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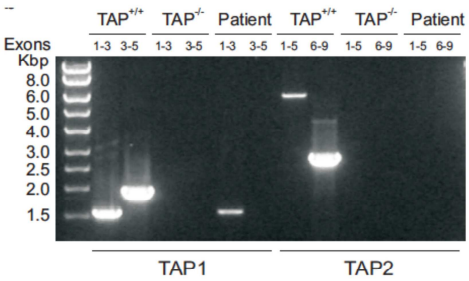

(A) RT-PCR of the different exons of TAP1 and TAP2 showing absence of TAP1 genes beyond exon 3 and complete absence of TAP2. RNA was extracted from BLCL of the patient, TAP-positive (T0) and TAP-negative (T2) control lines. Tapasin (*) was amplified as a loading control.
(B) PCR from genomic DNA showing presence of TAP1 up to exon 3 and absence of TAP2 in the same cell lines as (A).