CASE REPORT

Optimal approach to assessing T-cell function in haematopoietic cell transplant recipients

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SUMMARY

Standardised approaches to functional immune assessment after haematopoietic cell transplantation (HCT) are lacking. A 12-year-old girl with relapsed acute myelogenous leukaemia, 2 years post-unrelated HCT, underwent immunological evaluation prior to receiving live vaccinations. Assessment of standard immune parameters and T-cell proliferation to phytohaemagglutinin was reassuring. She was given *Varicella* vaccination based on usual post-transplant protocols but was hospitalised 10 days later with localised *Varicella* infection (vaccine strain). Following recovery, she underwent further assessment that showed reduced T-cell proliferation to an anti-CD3 stimulation panel (anti-CD3 alone, soluble anti-CD3⁺ anti-CD28 and soluble anti-CD3⁺ plus exogenous IL-2). On reassessment, 7 months later, T-cell responses to anti-CD3 stimulation were normal and she was revaccinated without further incident. Measurement of T-cell proliferation to anti-CD3 stimulants likely yields more useful information about global T-cell function and should be strongly considered prior to live vaccine administration post-allogeneic haematopoietic transplant.

total doses of methotrexate (5.6 mg). She had a complicated immediate post-transplant course, which included infections with *Clostridium difficile* and adenovirus. She also developed GVHD that manifested first as diarrhoea with mild GVHD on duodenal biopsy and was initially treated with ongoing tacrolimus and oral budesonide. Complete donor chimerism was achieved by 100 days post-HCT. Seven months post-transplant, she developed chronic GVHD with maculopapular rash involving <50% of skin surface and was treated with oral prednisone and subsequently briefly with rituximab. Tacrolimus was also continued at varying doses with a maximum of 1 mg twice daily, tapered down over several months and discontinued for the final time 573 days after transplant. Prednisone was started 207 days after transplant at a dose of 20 mg twice daily and was tapered down and discontinued 323 days after transplant. She had mild hypogammaglobulinaemia treated briefly with intravenous immunoglobulin. She was off of all immunosuppression at the time of her vaccination-readiness immune evaluation.

INVESTIGATIONS

Her immune assessment took place 728 days post-transplant (table 1). She had a normal total lymphocyte count ($4.57 \times 10^9/L$ (1.2–5.2)), CD3⁺ T cell count (3487 cells/ μL (865–3618)), CD4⁺ T cell count (1514 cells/ μL (497–2267)), CD16⁺ CD56 cell (NK cells) count (81 cells/ μL (69–691)), CD19⁺ B cell count (741 cells/ μL (106–1101)) and a slightly increased CD8⁺ T cell count (1644 cells/ μL (243–1303)). Her immunoglobulin levels showed slightly reduced IgG (429 mg/dL (503–1719)) with normal IgM (54 mg/dL (41–255)) and IgA (140 mg/dL (42–295)). Lymphocyte data were compared with an additional lymphocyte quantification data, which had taken place at an outside institution prior to her current assessment. This occurred 564 days post-transplant. At that time, she had a normal CD3⁺ T cell count (2601 cells/ μL (800–3500)), CD4⁺ T cell count (95 cells/ μL (400–2100)), CD8⁺ T cell count (1242 cells/ μL (200–1200)) and NK cell count (150 cells/ μL (70–1200)).

T-cell receptor excision circles (TREC) are a measure of thymic output and permit assessment of thymic reconstitution post-allogeneic HCT in age-appropriate individuals. Assessment of TREC at day 727 post-HCT revealed 6194 copies per million CD3⁺ T cells (reference range, >5270/

BACKGROUND

Several measures of adaptive immune function and reconstitution prior to live vaccination in haematopoietic stem cell transplant (HSCT) recipients are available but not widely used in the paediatric or adult haematology practices. This case provides an example of the relevance and necessity of a comprehensive immunological assessment prior to vaccination with live viruses, in the post-haematopoietic transplant setting.

CASE PRESENTATION

A 12-year-old girl with a history of relapsed acute myeloid leukaemia (AML) presented 2 years status post-unrelated haematopoietic cell transplantation (HCT) for immune evaluation and assessment of readiness to receive live vaccinations. At the age of 10 years, she received a matched unrelated bone marrow transplant. She received myeloablative conditioning with busulfan and cyclophosphamide along with immunosuppression with alemtuzumab, tacrolimus and methotrexate for prevention of graft versus host disease (GVHD). Alemtuzumab was discontinued shortly thereafter because of rash and fever thought to be due to it. She received three

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Table 1 Laboratory evaluations at presentation and follow-up

Test	At time of evaluation	Four-month follow-up	Seven-month follow-up	Range
CD45 ⁺ total lymph count, thou/ μ L	4.36	3.92*	4.65	1.28–5.84
%CD3 ⁺ (T cells)	80%	77%*	80%	61%–83%
CD3 ⁺ , cells/ μ L	3487	3013*	3705	865–3618
%CD4 ⁺ (T cells)	31%	31%*	30%	30%–56%
CD4 ⁺ (T cells), cells/ μ L	1350	1233*	1394	497–2267
%CD8 ⁺ (T cells)	38%	37%*	40%	16%–34%
CD8 ⁺ (T cells), cells/ μ L	1644	1446*	1856	243–1303
%CD19 ⁺ (B cells)	17%	21%*	18%	7%–27%
CD19 ⁺ (B cells), cells/ μ L	741	815*	822	106–1101
%CD16 ⁺ +CD56 ⁺ (NK cells)	2%	2%*	2%	3%–20%
CD16 ⁺ +CD56 ⁺ (NK cells), cells/ μ L	81	67*	91	69–691
CD4 ⁺ /CD8 ⁺ ratio	0.8	0.9*	0.8	>0.9
T-cell receptor excision circles	6194	†	8210	>5270
IgA, mg/dL	140	156	†	42–295
IgM, mg/dL	54	58	†	41–255
IgG, mg dL	429	488	†	503–1719
PWM %CD45 ⁺ lymphocytes	2.9%	2.4%	†	>4.5%
PWM %CD3 ⁺ T cells	3.3%	2.6%	†	>3.5%
PWM %CD19 ⁺ B cells	1.1%	2.2%	†	>3.9%
PHA %CD45 ⁺ lymphocytes	57.5%	59.0%	†	>49.9%
PHA %CD3 ⁺ T cells	63.5%	63.6%	†	>58.5%

Grey: abnormal laboratory.

*This testing was performed at 5 months' follow-up.

†Laboratory not performed at this time.

PHA, phytohaemagglutinin; PWM, pokeweed mitogen.

million CD3⁺ T cells). Lymphocyte proliferation to mitogens was notable for diminished responses to the weak T-cell mitogen, PWM (pokeweed mitogen) (%CD45 2.9% (>4.5%); %CD3 3.3% (>3.5%)) and adequate response to the more robust T-cell mitogen, phytohaemagglutinin (PHA) (%CD45 57.5% (>49.9%); %CD3 63.5% (>58.5%)). The normal TREC copies relative to the total T cell count and adequate response to PHA was reassuring for normal immune reconstitution at 2 years post-HCT. Based on this information, she was given live vaccination with measles, mumps and *Rubella* (MMR) vaccine and *Varicella* vaccine (Varivax 0.5 mL). She returned to an outside facility 10 days later with localised rash consistent with *Varicella* infection, subsequently confirmed to be vaccine strain (oka/Merck strain).

Given the infection post-vaccination, she was re-evaluated immunologically 4 months later. This time, additional functional T-cell studies were conducted, including lymphocyte proliferation to anti-CD3 stimulants. Cross-linking of the CD3 coreceptor with or without additional costimulation is considered a more physiological, yet global measure of T-cell function. Patient peripheral blood mononuclear cells were cultured with soluble anti-CD3 alone, anti-CD3⁺ anti-CD28 and anti-CD3⁺ IL-2 (figure 1A). Lymphocyte proliferation to standard mitogens, PHA and PWM were also repeated. She had similar responses to PHA (%CD45 59% (>49.9%); %CD3 63.6% (>58.5%)) and PWM (%CD45 2.4 (>4.5%); %CD3 2.6% (>3.5%)). She had a normal response to soluble anti-CD3 (%CD45 19.4% (>19.4%); %CD3 24.3% (>20.3%)); however, in the presence of anti-CD3⁺ anti-CD28, there was significantly reduced response (%CD45 18.1% (>37.5%); %CD3 22.1% (>44.6%)). IL-2 is a powerful T-cell growth factor, and when the patient's T cells were stimulated with anti-CD3⁺ IL-2, the expected robust response was not observed. On the contrary, there was a minimal

reduction in proliferation (%CD45 39.3% (>41.7%); %CD3 44.4% (>46.2%)). Based on this result, further vaccination was deferred at the time of this evaluation.

OUTCOME AND FOLLOW-UP

The patient was reassessed 7 months after the initial evaluation (day 943 post-HCT). Lymphocyte proliferation studies were repeated with anti-CD3 panel of stimulants (figure 1B). This time, there were normal responses to anti-CD3 (%CD45 36.9% (>19.4%); %CD3 44.1% (>20.3%)), anti-CD3 combined with anti-CD28 (%CD45 47.9% (>37.5%); %CD3 54.7% (>44.6%)) and anti-CD3 combined with exogenous IL-2 (%CD45 46.9% (>41.7%); %CD3 53.3% (46.2%)). Given her improved T-cell functional studies, she was given a second dose of the measles, mumps, *Rubella* and *Varicella* in the form of the combined vaccine and tolerated it well.

DISCUSSION

Varicella vaccine and MMR are the live vaccines currently recommended for post-HSCT patients. Based on current guidelines, the minimum recommended requirements for live vaccination in this population indicates that such vaccines be administered at 24 months post-transplant, with no evidence of GVHD, no immunosuppression and no intravenous immunoglobulin (IVIG) treatment for 8–11 months (depending on dose).^{1 2}

Immune reconstitution after HCT is highly variable. Innate immunity recovers quickly; however, adaptive immunity, especially for T and B cells, can take months to years to recover.³ CD4⁺ T cells generally take the longest to recover, and this can be delayed by GVHD and the therapies used for treatment.⁴ The type (related, unrelated, haploidentical etc) and source (bone marrow, peripheral blood stem cells, cord blood) of donor

Figure 1 Lymphocyte and T-cell proliferative testing at 4 months after initial evaluation (A, left) and 7 months after initial evaluation (B, right).

haematopoietic cells can affect immune reconstitution as well.⁵ Poor T-cell regeneration and thymic function as evaluated by TREC copies are associated with poor outcomes in the paediatric post-HCT population.⁴ T-cell reconstitution is strongly linked to outcomes and prevention of complications after HCT such as opportunistic infection, GVHD as well as relapse of the initial disease.⁶ Furthermore, imbalances or persistence of specific populations of T cells over others (effector CD4⁺ T cells compared with T regulatory cells) has been associated with the development of chronic GVHD.⁷ New strategies have been and are currently being explored to help enhance immune reconstitution after HCT.^{5,6}

There is great variability in clinical practice in the timing and desired criteria for live vaccination post-HCT.^{8,9} This is illustrated in the review by Forlenza and Small, which included the only four available studies on *Varicella* vaccine following HCT, of which only three provided their criteria for vaccination.¹⁰ The only criteria used consistently across all three studies were CD4⁺ T cell level count >200 cells/ μ L and absence of exogenous immunosuppression. Other criteria used in some but not all studies included an IgG level >500 mg/dL (1/3 studies), PHA response within the lower limit of normal (1/3 studies) and CD3⁺ T cell count >700 cells/ μ L (1/3 studies), lymphocyte count >1000/ μ L, normal antibody response to inactivated polio vaccine, normal B-cell and T-cell responses to tetanus toxoid, and response to greater than one non-live vaccine post-transplant.¹⁰ Similarly, the response rate to the vaccine (seroconversion rate) was highly variable and ranged from 55% in the largest study to 88.8% in the smallest study. A more recent study examined the response of 31 post-HSCT children to a high-titre zoster vaccine (generally considered to be contraindicated in post-HCT patients). Eighteen patients seroconverted after vaccination while 13 remained seronegative.¹¹ A number of immune parameters were measured and compared between those that seroconverted and those that did not.¹¹ There were no statistically significant differences in the two groups in regards to timing of vaccination, previous *Varicella* status, lymphocyte count, IgG level, CD4⁺ T cell count, CD4/8 ratio or proliferative response to PHA. This further demonstrated that these measures do not seem to adequately

assess readiness or predict response to vaccination. Recently, Miller *et al* conducted a survey of UK National Health Service providers who provide care for post-HCT patients.¹² Only 14/27 adult providers and 2/37 providers overall reported giving MMR and *Varicella* vaccinations as part of the post-HCT routine vaccination programme, respectively. In those that administer these vaccines, respondents noted they delay live vaccinations for mild (67%) or moderate to severe (31%) GVHD, any immunosuppressive therapy (97%), acute illness (81%), CD4⁺ T cell count <200 cells/ μ L (50%), anti-CD20 antibody treatment in the past 6 months (47%) and IVIG in the past 6 months (31%).¹²

This patient had an initial immune evaluation 2 years after transplant, which was reassuring for apparently normal immune reconstitution. However, she developed localised *Varicella* infection soon after vaccination. On follow-up, she demonstrated evidence of impaired T-cell function when using a more sensitive and physiological measure of global T-cell responsiveness. The first result suggested that she likely had suboptimal T-cell function, which increased her risk for developing an infection when exposed to a live, attenuated vaccine. Serial assessment 7 months later demonstrated improvement in T-cell function, and subsequent vaccination was without incident or further infection. Lymphocyte stimulation with PHA, a powerful T-cell stimulant, and PWM, a B-cell and weak T-cell stimulant, provides a general non-specific assessment of T-cell function; these plant lectins activate T cells in a polyclonal manner by cross-linking glycoproteins on the cell surface. However, measurement of T-cell function using cross-linking of the CD3 coreceptor, with or without additional costimulation, via CD28, or providing a potent T-cell growth factor, like IL-2, offers more physiological insight into T-cell responsiveness. This patient was at somewhat higher risk of poor immune reconstitution and skewed T-cell receptor repertoire due to significant immune suppression for her acute and chronic GVHD and early adenovirus reactivation; thus, this test may not be needed in the majority of patients. Nonetheless, this test may be a more reliable and sensitive indicator of T cell function in transplant patients and could be considered in assessing readiness for live vaccination in these patients, especially those at increased risk.

Learning points

- ▶ Standardised clinical approaches to functional immune assessment after HCT are lacking, especially as it pertains to live vaccination.
- ▶ This patient had apparently reassuring immune reconstitution assessment 2 years after transplant; however, she still went on to develop localised *Varicella* infection shortly after vaccination.
- ▶ Phytohaemagglutinin and pokeweed mitogen are normally used to provide global polyclonal assessment of T-cell function but may not always be indicative of more subtle impairment in T-cell responsiveness.
- ▶ Anti-CD3 stimulation of T cells (including anti-CD28 or IL-2) provides a global yet more physiological assessment of T-cell function. This may, therefore, be a more suitable method of functional immune assessment prior to live vaccination.

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