Video Article

Development of an IFN- γ ELISpot Assay to Assess Varicella-Zoster Virus-specific Cell-mediated Immunity Following Umbilical Cord Blood Transplantation

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Abstract

Varicella zoster virus (VZV) is a significant cause of morbidity and mortality following umbilical cord blood transplantation (UCBT). For this reason, antiherpetic prophylaxis is administered systematically to pediatric UCBT recipients to prevent complications associated with VZV infection, but there is no strong, evidence based consensus that defines its optimal duration. Because T cell mediated immunity is responsible for the control of VZV infection, assessing the reconstitution of VZV specific T cell responses following UCBT could provide indications as to whether prophylaxis should be maintained or can be discontinued. To this end, a VZV specific gamma interferon (IFN- γ) enzyme-linked immunospot (ELISpot) assay was developed to characterize IFN- γ production by T lymphocytes in response to *in vitro* stimulation with irradiated live attenuated VZV vaccine. This assay provides a rapid, reproducible and sensitive measurement of VZV specific cell mediated immunity suitable for monitoring the reconstitution of VZV specific immunity in a clinical setting and assessing immune responsiveness to VZV antigens.

Video Link

The video component of this article can be found at <http://www.jove.com/video/51643/>

Introduction

First performed in 1989, UCBT is increasingly used as part of the treatment of various neoplastic and nonneoplastic blood disorders in children¹. VZV is a cytopathic human alphaherpesvirus which causes two different diseases, varicella (after primary infection) and herpes zoster (after reactivation). Following primary infection, VZV persists throughout the life of the host sheltered within sensory nerves of dorsal root ganglia. One of the most threatening infectious complications following UCBT is associated with VZV^{2,4}. In our clinical center, in absence of VZV prophylaxis, the cumulative incidence of VZV disease at 3 years postUCBT was 46%². In these patients, *de novo* infection with or reactivation of VZV is often associated with visceral dissemination to the central nervous system, lungs and liver⁵⁻⁷. As a result, acyclovir, valacyclovir or famciclovir prophylaxis is commonly administered to UCBT recipients^{8,9}. However, this treatment strategy does not take into account the protective potential of VZV specific T lymphocytes or the kinetics of reconstitution of VZV specific T cell responses. Potential problems associated with the expanding use of long term antiherpetic prophylaxis include a) patient overtreatment; b) the development of antiviral drug resistance^{10,11}; and c) impairment of VZV specific immune reconstitution^{12,13}. Because detection of functional VZV specific T lymphocytes correlates with the presence of long term protection from VZV infection and improved clinical outcome^{4,14,15}, monitoring cell mediated immune responses directed against VZV during the posttransplant period might result in a more rational use of antiviral treatment by enabling medical practitioners to distinguish patients who would benefit from VZV prophylaxis from those whose immune system is capable of controlling VZV replication^{4,13}.

The IFN- γ ELISpot assay is widely used for the monitoring cell mediated immune responses in a variety of experimental systems and clinical conditions. Spots are generated following the cleavage of a chromogenic substrate, generating a visible and stable precipitate at the site of the reaction. Each individual spot thereby represents the footprint of an individual cytokine-producing cell. IFN- γ ELISpot not only measures the ability of individual cells *ex vivo* to produce IFN- γ in response to *in vitro* stimulation with cognate antigen, but it also provides an estimate of the frequency of responding cells in a given cell population^{16,17}. In addition to its high sensitivity, IFN- γ ELISpot is straightforward to perform, making its use possible in the context of personalized clinical protocols aimed at guiding initiation or cessation of antiviral treatment. The procedure

detailed below describes an ELISpot assay that is specifically designed to detect and measure the production of IFN- γ by peripheral blood mononuclear cells following *in vitro* stimulation with VZV derived antigens.

Protocol

This research protocol was approved by the Institutional Ethics Review Board of CHU Sainte-Justine, Montreal, Quebec, Canada, where the study was conducted. Informed consent was sought and obtained from all study participants, their parents or legal guardians. All procedures performed on days 1 and 2 must be carried out under sterile conditions (*i.e.* under a laminar flow hood). Standard safety procedures for handling human blood should be strictly observed.

1. Coating the Plates

1. Permeabilize the polyvinylidene difluoride (PVDF) membranes at the bottom of 96 well MultiScreen IP white ELISpot plates by adding to each well 20 μ l of 35% ethanol for 1 min. Membranes should become slightly translucent.
2. Immediately wash the wells 3 times with 200 μ l of 1x phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4) using a multichannel pipette or similar device. This step is critical given that ethanol residues can affect cell viability and binding of capture antibody. ELISpot plates are fragile and should be handled carefully: the use of an automated plate washer is not recommended.
3. Coat the wells with 10 μ l of purified mouse anti-human IFN- γ capture antibody diluted in 1x PBS to a final concentration of 10 μ g/ml. Cover the plates with regular plastic wrap and incubate overnight at 4 °C.

2. Blocking Plates

1. Empty the wells, tapping them dry, and wash plates 5 times with 200 μ l of 1x PBS.
2. Fill the wells with 200 μ l of complete RPMI 1640 medium and incubate plates for 2 hr at 37 °C (blocking step).
3. Wash the plates 5x with 200 μ l of 1x PBS and keep the wells full of 1x PBS until cells are ready to be plated.

3. Cell Plating and Stimulation

1. Prepare peripheral blood mononuclear cells (PBMC) from human venous blood samples by centrifugation on Ficoll-Paque gradients using standard procedures. Alternatively, using standard methods, thaw aliquots of PBMC frozen under liquid nitrogen. After the last centrifugation, resuspend cells at a final concentration of 2×10^6 cells/ml and incubate overnight at 37 °C under a 5% CO₂ atmosphere in a water jacket incubator.
2. Remove PBMC from the incubator and resuspend them in a final volume of 5 ml of complete RPMI 1640 medium.
3. Incubate PBMC in the presence of 10 μ l of genetically engineered endonuclease from *Serratia marcescens* for 5 min at room temperature.
4. Remove a 50 μ l aliquot of PBMC and mix with 50 μ l of trypan blue dye. Count cells and estimate % viability using a haemocytometer and microscopic examination (dye exclusion method). Various automated methods for cell counting and assessment of cell viability can also be used. PBMC should be >95% viable. Discard sample when viability is lower than 95%.
5. Centrifuge PBMC at 700 x g for 5 min, discard supernatant, and resuspend cells in AIM-V medium supplemented with 2% (v/v) inactivated human serum (IHS) at a final concentration of 2×10^6 cells/ml. Keep at 37 °C until stimulation mixtures are added to the plate.
6. Add to the wells, one drop at a time, 100 μ l of the appropriate stimulation mixture. Three stimulation mixtures should be used as described below:
 1. Use AIM-V medium supplemented with 2% (v/v) inactivated human serum (IHS) as negative control.
 2. Use anti-CD3 monoclonal antibody (clone OKT3) diluted in AIM-V medium supplemented with 2% (v/v) IHS to a final concentration of 0.5 μ g/ml as positive control.
 3. Use VZV antigen, in the form of either γ -irradiated (10,000 Gy; 30 min exposure time) live attenuated VZV vaccine (1,350 plaque forming units [PFU]/ml) diluted 1:200 in AIM-V medium supplemented with 2% (v/v) IHS or 1 μ g of an equimolar mixture of 67 peptides (15 amino acid residues) synthesized based on the sequence of the VZV immediately early (IE63) phosphoprotein. Control irradiation efficacy by monitoring the absence of visible signs of cytopathic effects after 4 days in PBMC cultures.
 4. Prepare all wells in duplicate.
7. Add to the wells, one drop at a time, 100 μ l of cells prepared in step 5.4 to the appropriate wells. Two wells should be left without cells (negative control). Incubate for 20 hr at 37 °C under a 5% CO₂ atmosphere in a water jacket incubator. Do not shake, move, or stack the plates on top of one another during incubation.

4. Spot Development and Detection

1. Discard the cells and wash the plates 10x with 1x PBS supplemented with 0.05% (v/v) Tween 20 (PBST). Tween 20 helps detach cells that have adhered to the PVDF membrane following overnight incubation.
2. Using a multichannel pipette, add 100 μ l of 0.5 mg/ml biotin conjugated anti-IFN- γ monoclonal antibody (4S.B3 clone) diluted in 1x PBS containing 0.5% (w/v) bovine serum albumin (BSA). Incubate plates for 2 hr at room temperature.
3. Wash the wells 6x with PBST and add each well 100 μ l of streptavidin conjugated with alkaline phosphatase diluted 1:1,000 in 1x PBS containing 0.5% (w/v) BSA. Cover the plates and incubate for 1 hr at room temperature.
4. Wash the plates 3x with PBST and 3x with 1x PBS. Add 100 μ l of a 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium chloride (NBT) substrate solution and incubate 5 min at room temperature.
5. Wash the plates under running distilled water. Remove the bottom section of the ELISpot plate and wash both sides of the membrane with distilled water. Air dry the plates.

6. Examine the wells and enumerate the spots using a stereoscopic dissection microscope or scan the plates using an automated spot counter. Keep the plates away from light if they cannot be examined on the same day.
 1. Average the numbers of spots counted in corresponding duplicate wells and subtract the number of spots counted in the negative control wells (no cells) from the number of spots counted in the test wells.
 2. Express IFN- γ production as the number of spot-forming units (SFU) per 10^6 PBMC.
 3. Consider that test samples are positive if the number of SFU is >50 per 10^6 PBMC and 2 standard deviations (SD) above the negative control (cells + AIM-V medium supplemented with 2% (v/v) IHS).
 4. Use a value of 400 SFU per well when spots are too numerous to be counted.
7. Test whether ELISpot data are normally distributed or not using the Kolmogorov-Smirnov test. When data are normally distributed, perform statistical comparisons using Student's t test (2 groups) or ANOVA and the Bonferroni post-test (multiple comparisons). If data are not normally distributed, use the Mann-Whitney U test (2 groups) or the Kruskal-Wallis test and Dunn's post-test (multiple comparisons).

Representative Results

The IFN- γ ELISpot protocol detailed above was developed and optimized in our laboratory to measure the magnitude and the quality of cell mediated immune responses directed against VZV⁴. Diverse sources of VZV antigen can be used for the stimulation step. These include: a) commercially available detergent inactivated extracts from VZV infected Vero cells¹⁸; b) pools of overlapping synthetic peptides from specific VZV encoded proteins, including IE63¹⁵ and ORF4¹⁹; c) live attenuated varicella zoster vaccine²⁰; and d) UV inactivated preparations of VZV antigens from the supernatant of disrupted VZV infected MRC cells¹⁴. In our case, dilutions of live attenuated VZV vaccine were preferred over cell culture derived VZV in order to insure uniformity in antigen source and preparation and to limit culture and manipulation of live wildtype VZV strains in a clinical setting where nosocomial transmission is a serious concern¹⁰. In addition, γ irradiation was preferred over UV inactivation²¹ because of the comparative precision with which doses of γ radiation can be delivered and because, unlike UV, γ -rays efficiently penetrate sealed VZV containing vials. Irradiation of viral stocks led to consistently higher estimates of the frequency of VZV specific IFN- γ producing cells in PBMC samples obtained from a healthy donor at all dilutions of VZV antigen tested (**Figure 1**). This is possibly the result of the mitigation of cytopathic effects induced by live attenuated VZV in cell culture.

To determine which dilution VZV antigen yielded maximal readouts in terms of numbers of SFU per 10^6 PBMC, IFN- γ ELISpot was performed using two-fold dilutions of γ irradiated live-attenuated VZV vaccine and PBMC samples obtained from a group of immunocompetent children aged between 9 months and 12 years who had a documented history of varicella and/or a positive serology for VZV ($n = 50$). Study subjects were enrolled at the Infectious Diseases Clinic and Special Immunology Clinic of CHU Sainte-Justine between June 2007 and September 2009. Statistically significant differences were observed between median frequencies of IFN- γ producing cells that were obtained using the 1:200, 1:400 and 1:800 dilutions of VZV antigen ($p < 0.0001$, Kruskal-Wallis test) (**Figure 2**). These disparities were accounted for by the difference between the 1:200 dilution (median = 115.0 SFU per 10^6 PBMC; interquartile range [IQR] = 75.0 - 232.5 SFU per 10^6 PBMC) and the 1:800 dilution (median = 50.0 SFU per 10^6 PBMC; IQR = 20.0 - 105.0 SFU per 10^6 PBMC), and between the 1:400 dilution (median = 92.5 SFU per 10^6 PBMC; IQR = 52.5 - 177.5 SFU per 10^6 PBMC) and the 1:800 dilution ($p < 0.001$ and $p < 0.01$, respectively, Dunn's multiple comparisons test) (**Figure 2**). For this reason, the 1:200 dilution of γ irradiated live-attenuated VZV vaccine was selected for use in routine measurements of VZV specific cell mediated immune responses.

To determine whether IFN- γ production resides in CD4⁺ and/or CD8⁺ T lymphocyte subsets, PBMC samples obtained from a healthy donor were subjected to depletion of CD4⁺ or CD8⁺ cells using anti-CD4 or anti-CD8 antibody coupled magnetic beads according to the manufacturer's instruction. In concordance with previously published reports^{22,23}, depletion of CD4⁺ cells resulted in a striking reduction in the frequencies of IFN- γ producing cells that were obtained using the 1:100, 1:200 and 1:400 dilutions of VZV antigen, while depletion of CD8⁺ cells did not lead to such a reduction and positive controls (stimulation with anti-CD3 monoclonal antibody) were not similarly affected (**Figure 3**). These results suggest that the majority of cells that produce IFN- γ in response to stimulation with VZV antigens are CD4⁺ T cells. Alternatively, these results could stem from depletion of CD4⁺ antigen presenting cells (*i.e.* monocytes, dendritic cells) from the PBMC pool, leading to reduced levels of presentation of VZV antigens to responder CD8⁺ T lymphocytes. The use of irradiated VZV is unlikely to have contributed to the skewing of the response towards CD4 dominance, as similar results were obtained by other groups using different techniques and antigen sources^{14,21,22}. A low magnification micrograph of representative ELISpot wells is presented in **Figure 4**.

Problem/issue	Possible causes	Remedy/solution
High background/Poorly-defined or confluent spots	High numbers of dead cells.	Assess cell viability before culture set-up and stimulation.
	Inadequate membrane pre-wetting steps.	Make sure to respect pre-wetting time and that membrane turn to gray after 1 min. 35%

		ethanol should be prepared immediately before use.
	Plates moved during the incubation period.	Do not move plates to avoid poorly-defined snail trail spots.
	Wash steps.	Manual wash is more efficient than plate washers.
	Formation of protein aggregates.	Filter both capture and detection antibodies to reduce background and false positive spots.
	Secondary and detection antibody concentration.	Adjust biotinylated secondary/detection antibody concentration to reduce background.
	Cold developing reagents.	Bring substrate to room temperature to avoid underdevelopment.
No spots/Blank wells	Poorly defined spots.	Do not stack plates during incubation period to have a homogeneous temperature in the different wells.
	Stimulation substrate not well prepared.	Bring the peptide mixture aliquot to room temperature for 15 min to avoid crystal formation.
	Cell clumping.	Resuspend cells into single-cell suspension to avoid underestimation of spot-forming units.
	Cells not stimulated appropriately.	Use a polyclonal activator as a positive control.
	Inhibition of enzyme reaction by tween-20.	Wash plates with PBS before adding BCIP/NBT substrate solution.
	Wrong antibody pairs.	Make sure that the capture and detection antibodies react with different antigenic epitopes.

Table 1. Additional troubleshooting.

Figure 1. Effect of γ -irradiation of live inactivated VZV vaccine on the frequency of IFN- γ producing cells as determined using IFN- γ ELISpot. VZV specific IFN- γ ELISpot was performed as described under Protocol Text using PBMC from a control volunteer and serial dilutions of γ irradiated (10,000 Gy) live attenuated VZV vaccine as antigen. Data represent the mean of duplicate measurements and error bars represent the standard error of the mean. PBMC: peripheral blood mononuclear cells; SFU: spot forming units; VZV: varicella zoster virus.

Figure 2. Quantification of VZV specific cell mediated immune responses in children with documented evidence of previous infection with VZV. VZV specific IFN- γ ELISpot was performed as described under Protocol Text using PBMC isolated from subjects with a history of varicella ($n = 50$) and serial dilutions of γ irradiated (10,000 Gy) live attenuated VZV vaccine as antigen. Horizontal lines represent the median, boxes represent interquartile range (IQR), and whiskers represent the range of values. PBMC: peripheral blood mononuclear cells; SFU: spot forming units; VZV: varicella zoster virus.

Figure 3. Effect of depletion of CD4⁺ or CD8⁺ cells on the frequency of IFN- γ producing cells as measured using IFN- γ ELISpot. Depletion of CD4⁺ or CD8⁺ cells and VZV specific IFN- γ ELISpot were performed as described under Protocol Text using PBMC from a control volunteer and serial dilutions of γ irradiated (10,000 Gy) live attenuated VZV vaccine as antigen. Data represent the mean of duplicate measurements and error bars represent the standard error of the mean. PBMC: peripheral blood mononuclear cells; SFU: spot forming units; VZV: varicella zoster virus.

Figure 4. Representative ELISpot wells. VZV specific IFN- γ ELISpot was performed as described under Protocol Text using PBMC from five control volunteers and serial dilutions of γ irradiated (10,000 Gy) live attenuated VZV vaccine as antigen. First row: negative controls (AIM-V medium supplemented with 2% (v/v) IHS); second row: positive controls (anti-CD3 monoclonal antibody); third row: VZV antigen. Numbers in the upper corners represent SFU per well as measured using an automated spot counter. PBMC: peripheral blood mononuclear cells; SFU: spot forming units; VZV: varicella zoster virus; IHS: inactivated human serum.

Discussion

Modifications and troubleshooting: IFN- γ ELISpot assays have been used to examine cell-mediated immune responses directed against a variety of microbial pathogens, including human immunodeficiency virus type 1 (HIV-1)^{24,25}, hepatitis C virus (HCV)^{26,27}, and *Mycobacterium tuberculosis*^{28,29}, just to name a few. Here we described the development of an IFN- γ ELISpot assay to measure cellular immunity against, with the hope of defining correlates of VZV specific immune reconstitution in recovering pediatric UCBT recipients.

Key characteristics of this assay are four-fold. First, it does not require prior *in vitro* expansion of T cells, which is time consuming. Second, it can be performed using cryopreserved PBMC samples, which is best to minimize interassay variability. In addition, cryopreservation and parallel processing are well adapted to longitudinal studies, which require that samples be processed in batches. Third, to prevent cell clumping encountered during thawing of PBMC, treatment with an ultrapure genetically engineered endonuclease from *Serratia marcescens* was used. Cell clumping upon thawing PBMC occurs more frequently when using blood samples that are left overnight at room temperature before processing. The incorporation of genetically engineered endonuclease from *Serratia marcescens* is known not to affect cell viability, expression of cell surface markers and response to stimulation with cognate antigen in terms of SFU since low levels of nuclease are used and the duration of exposure is short²². Fourth, γ irradiated live attenuated VZV vaccine was used to stimulate PBMC. It was preferred over cell culture derived VZV in order to insure uniformity in antigen dose and preparation and to overcome the need for manipulating live VZV in a clinical setting where the safety of patients and laboratory personnel is a major concern. Taken together, the modifications that were introduced in the VZV-ELISpot assay were intended to make it better adapted for use in a clinical environment.

Limitations of the technique significance with respect to existing methods: Straightforward, sensitive and reproducible methods are required in order to measure host cell mediated immune responses directed against microbial antigens in the context of clinical followup. IFN- γ ELISpot is a robust, reproducible and informative assay that can be performed on venous blood samples using relatively low tech equipment (centrifuges, incubators, dissection microscope). However, it does not readily permit the simultaneous detection of multiple cytokines and cell surface markers. Traditional *in vitro* T cell proliferation assays, which have classically been used for the enumeration of CD4⁺ and CD8⁺ antigen specific T cell responses, are labor intensive, lack sensitivity and tend to suffer from a high degree of interassay variability^{30,31}. On the other hand, cytotoxic T lymphocyte assays (CTL) based on the lysis of autologous targets loaded with ⁵¹Cr and limiting dilution analysis tend to depend on time consuming *in vitro* expansion steps to quantify antigen specific CD8⁺ T cells that are present at a low precursor frequencies^{32,33}. Flow cytometry based assays that measure cytokine secretion following antigenic stimulation (intracellular cytokine staining [ICS])^{34,35} can also be used to characterize antigen-specific immune responses. ICS permits the simultaneous detection of cytokine production and cell surface expression of phenotypic markers in single cells. The sensitivity of ICS compares to that of ELISpot, but it requires larger numbers of cells^{36,37}. Methods that make use of fluorescent class I or class II peptide-major histocompatibility complex (pMHC) oligomers (pMHC tetramers)³⁸⁻⁴⁰ are precise and enable multiparametric characterization of lymphocyte subsets based on expression of cell surface markers. However, pMHC tetramer staining requires prior knowledge of the patients' human leukocyte antigen (HLA) type, which can be cumbersome and limit patient enrolment into a given study. Another limitation of this technique is that pMHC tetramer staining primarily allows the detection of T cells expressing medium to high affinity T cell receptors (TCR)⁴¹. On the other hand, CD8⁺ T cells expressing low affinity TCRs that are not stained by pMHC tetramers can be readily detected using IFN- γ ELISpot⁴¹. In addition, pMHC tetramers can bind to so-called exhausted CD8⁺ T lymphocytes that are prevalent in chronic viral infections^{42,43}, thereby skewing the functional assessment of antigen specific cell mediated immune responses. Finally, pMHC tetramer staining and ICS typically require highly trained personnel, comparatively expensive reagents, sophisticated instruments, and dedicated facilities, making these techniques notoriously difficult to implement in a clinical follow up setting.

Future applications: Dual color ELISpot assays capable of simultaneously detecting the production of both IFN- γ and IL-2⁴⁴ and dual- and triple-color fluorospot methods⁴⁵ have generated significant interest, as they allow the assessment of the polyfunctionality of antigen specific T lymphocytes, which is an important feature of efficient cell mediated immune responses⁴⁶.

Critical steps within the protocol: Additional technical issues must be given careful consideration. Because many variables might affect spot quality (*i.e.* size and density) and number, adherence to the protocol is essential to achieve sensitive and reproducible results. The consistent use of specific brands of monoclonal antibodies (capture and detection), ELISpot plates with PVDF membranes, and 35% ethanol for membrane treatment prior to coating is of high importance. For example, overtreatment with ethanol can affect the performance of the assay and spot quality. To prevent obtaining uninformative wells (*i.e.* >300 spots), we recommend using no more than 2×10^5 PBMC per well. When faced with low frequencies of antigen specific T cells, assay sensitivity can be enhanced by prestimulating cells *in vitro* with cognate antigen or expand them with polyclonal activators such as phytohemagglutinin (PHA) for a defined period of time (typically 3-7 days)³⁸. However, the final optimized procedure for the VZV ELISpot assay presented herein does not include *in vitro* prestimulation and/or expansion steps. Number and quality of spots were similar over an incubation range of 16-21 hr. Beyond 21 hr of incubation, spots were often more diffuse and sometimes overlapped, making their enumeration more difficult. Moreover, moving the plates while the cells are incubating can lead to poorly defined snail trail spots that are likewise more difficult to count. Additional troubleshooting cues are provided in **Table 1**.

When using an automated spot counter, sensitivity and spot size gating are set using positive control (anti-CD3 monoclonal antibody) and negative control (AIM-V medium supplemented with 2% (v/v) IHS). The important characteristics to consider during spot enumeration are size and shape (**Figure 4**). The former allows one to distinguish T cell derived cytokine spots from background spots that should be ignored. Spot number provides an estimate of the frequency of VZV specific T cells present in a given PBMC sample, while spot size reflects how much IFN- γ is produced by each individual cells.

To our knowledge, this is the only IFN- γ ELISpot method that makes use of γ irradiated live attenuated VZV vaccine as antigen⁴. Panels of overlapping synthetic peptides (15-mers) have also been used as antigen, including mixture of peptides based on the amino-acid sequence of the VZV IE63 phosphoprotein. IE63 is highly immunogenic⁴⁷ and appears to be crucial for viral infectivity and the establishment of latency⁴⁸. IE63 specific T cells have been detected in healthy VZV seropositive donors¹⁵, which suggests that IE63 is an important target of cell mediated immunity directed against VZV. However, reactive cells were found to be largely CD4⁺ T cells¹⁵.

The VZV IFN- γ ELISpot assay was previously used by our group to assess the VZV specific T cell responses in 28 children who underwent UCBT to treat neoplastic or inherited blood disorders⁴. Results that were obtained during the course of this study suggested that both CD4⁺ and CD8⁺ T cell subsets were implicated in IFN- γ production and that reconstitution of the naive CD8⁺ T cell compartment led to more robust VZV specific cell mediated immune responses in terms of IFN- γ production. Importantly, these results also showed that the detection of more than 150 SFU per 10^6 PBMC was consistent with T cell mediated protection against VZV infection or reactivation⁴. However, this threshold value for VZV specific cell mediated immunity in the context of UCBT and ensuing immune reconstitution or lack thereof will have to be validated in larger prospective multicenter studies.

Disclosures

The authors declare that they have no competing financial interests.

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