EVALUATION OF AN *IN VITRO* T CELL ASSAY TO DETECT CELLULAR RESPONSES TO VARICELLA ZOSTER VIRUS (VZV)

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Abstract

Background: Varicella zoster virus (VZV) causes chicken pox and remains dormant in the host except during immunosuppression. Thus, immunocompromised individuals are at an increased risk for reactivating VZV. IL-2 and IFN-γ are cytokines required to induce a protective proliferation response of antigen specific T cells. This study was designed to evaluate an *in vitro* T cell assay that detects cellular immunity to VZV.

Methods: Whole blood from healthy donors who previously had chicken pox (VZV+ donors) and from naïve donors that have never had chicken pox (VZV– donors) were stimulated with VZV lysate or phytohemagglutinin (PHA) for 24 hours. Plasma was collected and assayed for INF-γ and IL-2 cytokine production using the Meso Scale Discovery (MSD) platform. Confirmation of the naïve status was confirmed by an immunoassay to detect IgG and IgM to VZV (Vidas, BioMerieux). A proliferation test on VZV+ donors detected the percentage of proliferating CD4+ and CD8+ cells in response to VZV after five days.

Results: Cytokine responses to VZV were higher in VZV+ donors by a factor of 6 (IL-2 production) and a factor of 17 (IFN-γ production) compared to VZV– donors. The PHA responses in all donors were similar. The percentage of proliferating cells in VZV+ donors were 3-11% in CD4+ cells and 1.7-3.5% in CD8+ cells.

Conclusions: The cellular response to VZV as measured by cytokine release was increased in the VZV+ donors compared to the VZV– donors. This data suggests that the production of cytokines may indicate protective immunity to reactivation of VZV. This assay would be useful for identifying individuals with decreased cellular immunity to VZV and determine the need for a VZV vaccine.

Introduction

- Human herpes virus, varicella zoster virus (VZV), causes life long infection
  - Primary Infection = Chicken Pox (fluid filled lesions containing large number of viral particles)
  - VZV becomes dormant within the dorsal root sensory ganglia.
  - VZV reactivates when human host T cell-immunity declines
  - Reactivated Infection = Zoster/shingles (localized vesicular rash)
  - Reactivation often associated with severe neuralgia after rash has resolved
- Adequate T cell-mediated immunity is critical to maintaining latency of VZV and prevent reactivation

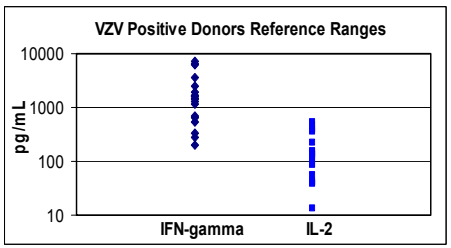
- **<u>Study purpose: Establish a laboratory method for the detection of VZV T-cell mediated immunity in healthy adults with a history of chicken pox but no recent history (within past year) of zoster.</u>**

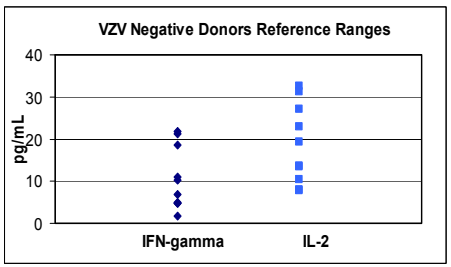
- T cell activation can be detected by measuring gamma interferon (IFN-γ) and interleukin 2 (IL-2) after stimulation with VZV antigens *in vitro*.
- Cytokine results are compared with detection of proliferating T cells after activation with specific VZV antigen.

Materials and Methods

- **Patients**
  - VZV Positive Donors (n=20) Healthy donors who previously had chicken pox.
  - VZV Negative Donors (n=2) Healthy donors who have never had chicken pox or the VZV vaccine.
- ***In vitro* Whole Blood Simulation**
  - Sodium Heparinized whole blood was collected and stimulated with either RPMI media, PHA (83µg/mL), or VZV (24µg/mL) for 24 hours.
  - Plasma was collected stored at -20°C for cytokine analysis.
- **Cytokine Analysis**
  - Human plasma was analyzed for cytokine production using the MSD multiplexed assay. Each well contained 7 Th1/Th2 cytokines. Plasma was incubated in the wells followed by application of a detection antibody. Read buffer was then applied and wells were read in the MSD Sector Imager.
- **Proliferation Assay**
  - PBMCs were isolated from CPT tubes and resuspended to 2 x 10^6 per mL. Cells were stained with CFSE, washed and resuspended in RPMI with 40% autologous plasma. Cells were stimulated with RPMI, PHA (2.5µg/mL, or VZV (24µg/mL) and incubated for 5 days. Following incubation, the stimulated cells were stained with CD4 PE, and/or CD8 FITC. Cells were analyzed on the Beckman Coulter FC500.

Reference Range Results





<u>VZV Donors Reference Range</u>

IFN-γ:Negative Range = 1.66pg/mL – 21.78pg/mL

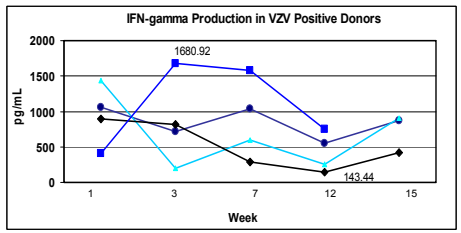
Positive Range = 203.47pg/mL – 7143.55pg/mL

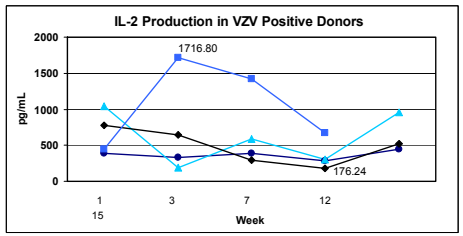
IL-2: Negative Range= 7.7pg/mL – 32.64pg/mL

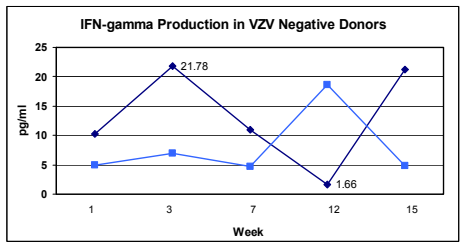
Positive Range = 13.34pg/mL – 532.70pg/mL

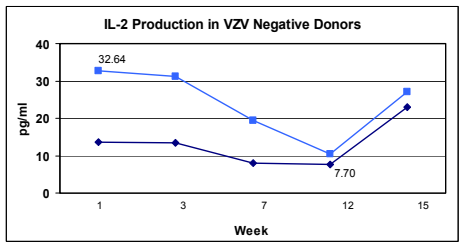
Whole Blood Stimulation with VZV Cell Lysates

Whole blood from VZV+ donors (n=4) that produced mid-reference range quantities of IFN-γ were stimulated with VZV antigen over a period of 15 weeks. Two VZV- donors were identified and also tested over 15 weeks. Plasma was collected after each stimulation and used to assess Th1/Th2 cytokine production.



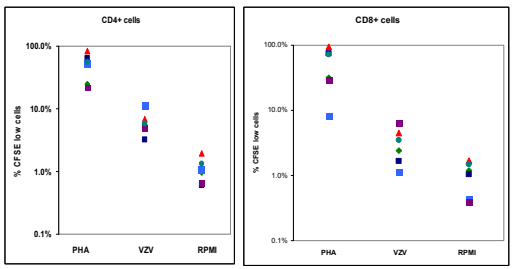






- **IFN-γ and IL-2 results were the most informative data collected from cytokine analysis. Other cytokine information was obtained for IL-4, IL-5, IL-10, IL12, IL13 (data not shown).**
- **The 5 VZV Positive Donors produced at least 100pg/mL more of IFN-γ and IL-2 than the VZV Negative Donors.**

T Cell Proliferation



- ***In vitro* stimulation of PBMCs with VZV lysate resulted in proliferation of both the CD4+ and CD8+ populations**

Conclusions

- ***In vitro* stimulation of whole blood indicates that cytokine levels vary over time. However, VZV positive donors consistently display higher levels of IFN-γ and IL-2 when compared to VZV negative donors.**
- **VZV negative donors had normal cytokine responses when stimulated with PHA (data not shown).**
- **Further research will determine if cytokine production and/or T cell proliferation can predict if an individual has adequate cell-mediated immunity to prevent VZV reactivation or if vaccination would be recommended**

References

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2. Boeckh M, Kim HW, Flowers MED, Meyers JD, and Bowden RA. Long-term acyclovir for prevention of varicella zoster virus disease after allogenic hematopoietic cell transplantation-a randomized double-blind placebo-controlled study. *Blood*. 2006. 107:5

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