From the Diagnostic Immunology Laboratories

IN THIS ISSUE:

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Spring Meetings

Please stop by and say "hello" in May at this year's American Society of Pediatric Hematology/Oncology (**ASPHO**) Annual Meeting in New Orleans as well as the Clinical Immunology Society (**CIS**) Annual Meeting in Chicago. We will be there with the Molecular Genetics Laboratory as the integrated Diagnostic Center for Heritable Immunodeficiencies.

We congratulate Virgil Villanueva on his retirement and thank him for all his wonderful years of service!
(pictured here, left to right, with coworkers, Barb Wanstrath, Lindsay Dunn, Carrie Gifford, department AVP, Sue Laupola, and Laboratory Supervisor, Darryl Hake)

THIS ISSUE'S FOCUS – CD45 RARO

We are very pleased to officially introduce our revised CD45 RARO panel. The assay was modified to include singlet gating, CD45, CD27, CCR7 and CD31 to enable identification of subpopulations of Naïve and Memory/Effector cells.

Naïve T lymphocytes are resting T cells that have not yet encountered antigen. Naïve T cells are present in both the CD4 and CD8 subsets. In CD4+ T cells, the naïve cell population can be further divided into those which are recent thymic emmigrants (CD31+) and those which proliferate post-thymically but have not yet encountered antigen (CD31-)[1]. See Figure 1.

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Memory T cells are long-lived antigen-specific T cells that have the capacity to quickly differentiate to end stage effector cells upon re-exposure to antigen. Memory T cells can be further divided into Central Memory and Effector Memory populations. Central Memory T cells (Tcm) express homing receptors, such as CCR7, that allow the cells to migrate to secondary lymphoid organs versus nonlymphoid tissue. Effector Memory T cells (Tem) are characterized by the presence of immediate effector function. Central and Effector memory T cells are present in both the CD4 and the CD8 subsets[2]. See Figure 2 for examples of Tem and Tcm populations

TEMRA cells (T Effector-Memory cells with reacquired RA) are a subset of very mature effector memory cells that have reacquired RA, and carry the largest amount of perforin of any of the effector cells[2]. Our previous methodology would have classified these as "Naïve," but with our additional markers, we are now able to identify the TEMRA cells (Figure 3). Using eight-color flow cytometry, monoclonal antibodies against antigens characteristic of each of the above populations are used to quantitate the relative proportions of each subset in the peripheral blood.

1. "Life after the thymus: CD31+ and CD31- human naïve CD4+ T-cell subsets" Blood, 22 January 2009, Vol. 113, No. 4, pp769-774.
2. "Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance." Annual Review of Immunology, 2004, 22:745-763

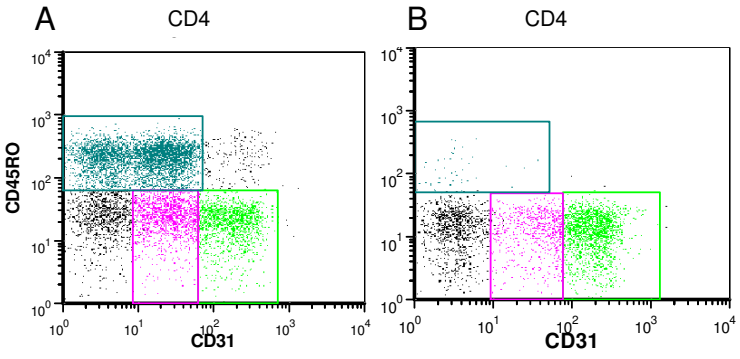

Figure 1. Typical differences in CD4 cell populations of an adult (A) versus a pediatric sample (B). The CD45RO-CD31bright are recent thymic emmigrants while the CD45RO-CD31dim are cells that have proliferated but not yet encountered antigen. CD45RO+ are memory cells.

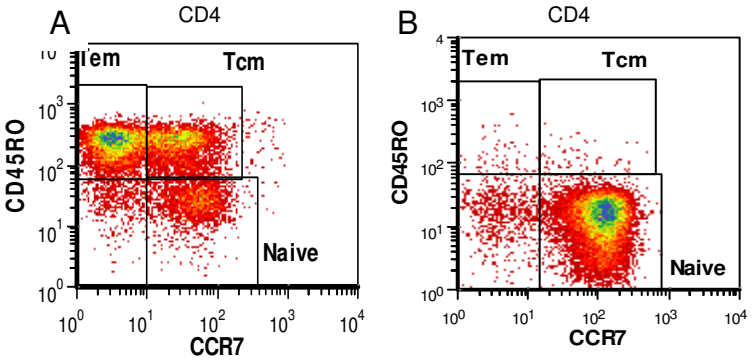

Figure 2. An adult (A) and a pediatric sample (B) illustrating the CD4 Tem and Tcm population changes with age. Tcm cells are CCR7+.

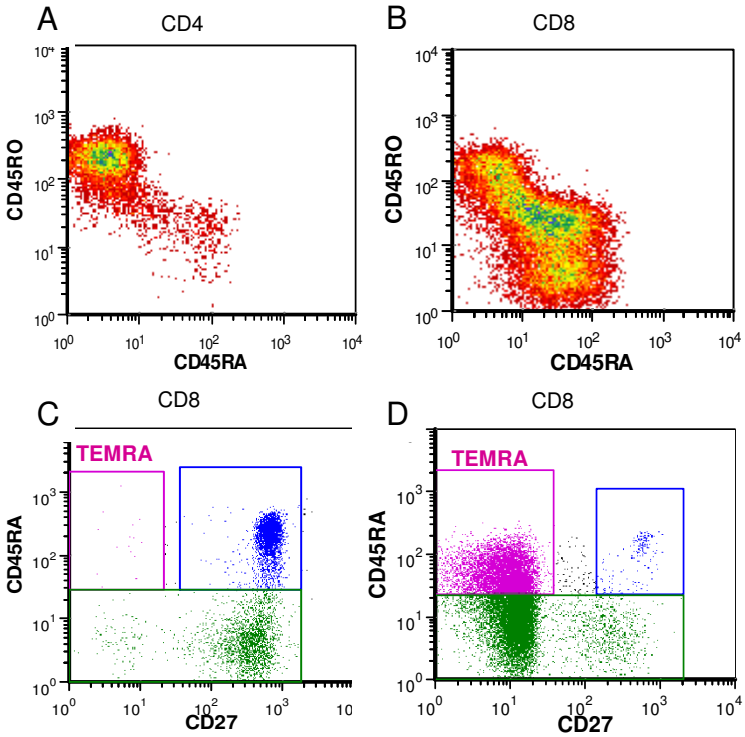

Figure 3. CD8 TEMRA cells (memory cells that have reacquired RA) are usually a small proportion of total memory cells. An adult control (C) is shown exhibiting few CD8 TEMRA. Using our old methodology a patient would have been classified as having a high percentage of Naïve CD8 (B), but by using the new gating strategies and antibodies in the revised panel, we more accurately classify this patient's CD8 population as TEMRA cells (D). Note the difference in this patient's CD4 (A) and CD8 (B) compartments.

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WASP Transplant Panel

Many may already be familiar with our Wiskott-Aldrich Syndrome Protein (WASP) Screening Assay, in which we compare WASP expression on patient lymphocytes against normal peripheral blood (PB) control lymphocytes. In this assay we report a ratio of the mean channels (MC, or intensity of staining). It is a very useful assay to serve as a screen for potential disease state (see Figure 4).

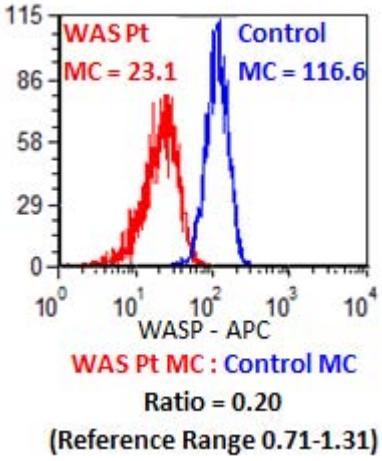

Figure 4. Histogram of a WAS patient overlaid with the PB control showing very descreased WASP expression.

The WASP Transplant Panel is intended to monitor the absence or otherwise atypical staining pattern of intracellular WASP after Bone Marrow Transplantation (BMT). Success of engraftment after BMT can be evaluated by performing multi-color flow cytometric analysis using monoclonal antibodies to cell surface antigens to characterize the hematopoetic cell populations of interest before the intracellular staining for WASP. We can, therefore, indicate the disease, carrier state, or mixed chimerism after BMT.

We look at the WASP expression on CD4+ and CD8+ T cells, NK cells, B cells, Monocytes, and Granulocytes. If we note chimeric expression, we include the percent of each cell lineage expressing or not expressing the WASP.

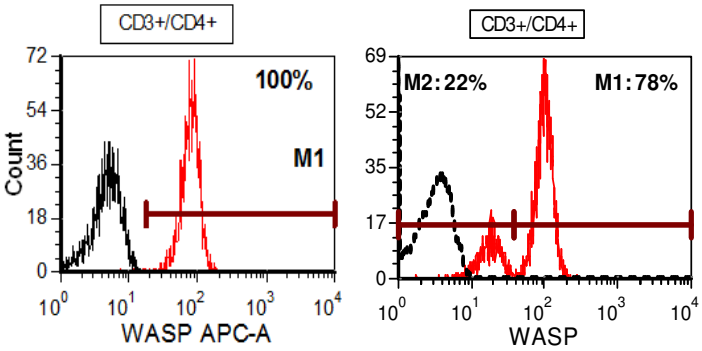

Figure 5. WASP expression on CD4+ T cells on a healthy control (left) and a WAS Patient Post-Transplant with mixed donor chimerism (right). Note the 78% donor cells in Marker 1 (M1) and 22% recipient in Marker 2 (M2).

Patient Vignette:

An infant male, who presented at another institution with petechiae at birth as well as occasional eczema and blood in stool, was diagnosed with WAS at four months of age. He underwent a preparative regimen of Bu/Cy/ATG and was transplanted with a 10/10 cord blood (male donor). He had minimal problems during his transplant hospital course. At Day +28 he was found to be only 30% donor. Immune suppression (cyclosporine and MMF) was rapidly weaned off, and he has since continued to have increasing engraftment, with total engraftment now 90% donor. A WASP transplant panel was performed and expression was very encouraging with a good percentage of all T, Myeloid, and NK cells expressing WASP (Figure 5). The current plan is to continue monitoring and comparing total engraftment and WASP Transplant expression monthly until stable. Lymphocyte Subsets, CD45 RARO, and Mitogen Stimulation are also regularly ordered to observe T cell reconstitution. With evidence of increasing engraftment, the family was reminded to monitor for signs of GVHD.

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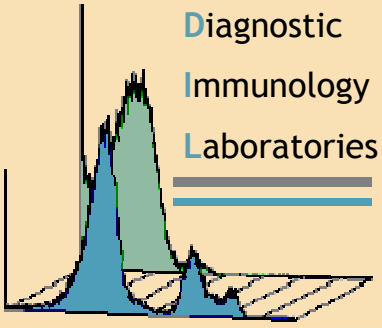
The Diagnostic Immunology Laboratories, consisting of the Clinical Immunology Laboratory and the Research Immunology Laboratory, are committed to providing the highest quality, comprehensive clinical testing available to aid in the detection, diagnosis and treatment of pediatric immunologic, as well as oncologic and hematologic, disorders. We're committed to applying scientific advances to promote efficiency, enhance patient care and improve clinical utility.

The clinical diagnostic laboratories are in compliance with all major regulatory agencies including CLIA (Clinical Laboratory Improvement Amendments), CAP (College of American Pathologists), HCFA (Health Care Financing Administration), HIPAA (Health Insurance Portability and Accountability Act) and JCAHO (Joint Commission on Accreditation of Healthcare Organizations).

The current menu of immunologic assays and information regarding shipping instructions is published on the last page of this Newsletter. The accompanying Test Requisition Form can be obtained through our website. Previous editions of the Newsletter can also be found at this website: www.cchmc.org/DIL

CONTACT US

Please visit our website or call us with any inquiries:
Ph: 513-636-4685
Fax: 513-636-3861
www.cchmc.org/DIL



New Tests *Now* Available:

- Cytokine Panel, now available on peripheral blood AND CSF
  Cytokines included are:
  - IL-1B - IL-2
  - IL-4 - IL-5
  - IL-6 - IL-8
  - IL-10 - IFN-g
  - TNF-a - GM-CSF
- Neopterin, peripheral blood and CSF

New Tests Down The Pipeline:

- B Cell Panel (new markers!)
- Campath – Plasma Levels
- Extended Mitogen Panel (PHA, PMA Calcium Ionophore at three concentrations, IL-2, CD3/CD28
- Restimulation Induced Cell Death (RICD), complements Fas-mediated Apoptosis assay

Feedback:

We would like to hear from our Clients. We invite you to share your questions and comments with us. Feel free to send/fax/email your comments to us: Fax 513-636-3861; Email: immunodeficiencies@cchmc.org

Cincinnati Children's Hospital
New Liberty Campus Location

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Current Menu of Available Tests:

Cincinnati Children's
change the outcome

Diagnostic Immunology Laboratory
Ph: 513-636-4685 Fax: 513-636-3861
www.cchmc.org/DIL

Test Requisition Form - 050112

Send to: **Julie Beach-Hematology/Oncology R2328**
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229-3039
MUST be received within 24 hrs of being drawn.
Maintain all samples, unspun, at room temperature.
Use Diagnostic Specimen packs and **FIRST OVERNIGHT PRIORITY SHIPPING** to ensure timely delivery.
The lab operates M-F only. Closed Saturday and Sunday.

Please call with the courier and tracking number of the package.

PATIENT & SAMPLE INFORMATION

Patient Name	Patient Identification Number	Date of Sample	Time of Sample
Date of Birth	Gender: ☐Male ☐ Female	Has the patient undergone BMT? ☐ No ☐ Yes – date of BMT	
Diagnosis or reason for testing			Diagnosis Code
Race: ☐ African-American ☐ American Indian ☐ Asian ☐ Caucasian ☐ Hispanic ☐ Other (specify) _____			
Medications:			

TESTS REQUESTED

☐ ALPS Panel	3ml (1ml) EDTA **CBC/Diff**[3]	☐ MHC Class I & II	3ml (1ml) EDTA
☐ Antigen Stimulation	10ml (5ml) Sodium Heparin[1]	☐ Mitogen Stimulation	10ml (5ml) Sodium Heparin[1]
☐ Apoptosis (Fas-mediated)	20ml (10ml) ACD-A	☐ Neopterin	3ml (1ml) EDTA, or CSF[3]
Note: Sample must be <24 hrs old and received Thursday AM		☐ Neutrophil Adhesion Markers(CD18/CD11b)	3ml (1ml) EDTA
☐ B Cell Panel	3ml (1ml) EDTA **CBC/Diff**[2]	☐ Neutrophil Oxidative Burst	3ml (1ml) EDTA
☐ BAFF, Plasma Levels	3ml (1ml) EDTA	☐ NK Function	10ml (5ml) Sodium Heparin[1]
☐ CD40L / ICOS	5ml (3ml) Sodium Heparin	☐ Perforin/Granzyme B	3ml (1ml) EDTA
☐ CD45RA/RO*NEW REVISED PANEL*	3ml (1ml) EDTA	☐ PNH Screen (FLAER/CD59)	3ml (1ml) EDTA
☐ CD52 Expression	3ml (1ml) EDTA	☐ pSTAT5	3ml (1ml) EDTA
☐ CD64 (Leuko64)	1ml (0.5ml) EDTA	☐ SAP (XLP1)	3ml (1ml) Sodium Heparin
☐ CD107a Mobilization	10ml (5ml) Sodium Heparin[1]	☐ Soluble CD163	2ml (1ml) EDTA
Note: CD107a is a 2 day test and cannot be accepted on Fridays		☐ Soluble IL-2R	3ml (1ml) EDTA
☐ CD127 / CD132	3ml (1ml) EDTA **CBC/Diff**[3]	☐ Sorted Engraftment	call to schedule
☐ CTL Function	10ml (5ml) Sodium Heparin[1]	☐ TCR α/β / TCR γ/δ	3ml (1ml) EDTA
☐ Cytokines, Intracellular	3ml (2ml) Sodium Heparin	☐ TCR V beta Repertoire	3ml (2ml) EDTA
☐ Cytokines, Plasma or CSF	5ml (3ml) EDTA, or CSF[3]	☐ WASP	3ml (1ml) Sodium Heparin
☐ EBV Immortalized Cell Line	3ml Sodium Heparin	☐ WASP Transplant Monitor	3ml (1ml) Sodium Heparin
☐ Check here if this is a research sample; signed consent required		☐ XIAP (XLP2)	3ml (1ml) EDTA
☐ Foxp3	3ml (1ml) EDTA **CBC/Diff**[2]	☐ ZAP-70 (only for SCID)	3ml (1ml) EDTA
☐ iNKT	3ml (1ml) EDTA	☐ Other _____	
☐ Lymphocyte Activation Markers	3ml (2ml) Sodium Heparin		
☐ Lymphocyte Subsets	3ml (1ml) EDTA		

Notes:
1. Volumes requested assume a normal absolute lymphocyte count (ALC). If the ALC is abnormal, please call the lab for adjusted volume requirements when ordering any of the following tests: Antigen or Mitogen Stim, CTL or NK Function, or CD107a.
2. Results of a same day CBC/Diff must accompany the sample where indicated (used to report absolute cell counts).
3. Neopterin or Cytokine CSF samples should be shipped at 2-8°C. Neopterin/Plasma Cytokine EDTA samples should be shipped at room temperature.

IN THE UPCOMING ISSUE:

- Apoptosis – Restimulation Induced Cell Death (RICD)

FOCIS Federation of Clinical Immunology Societies www.focisnet.org

jmcn Jeffrey Modell Centers Network

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