From the Diagnostic Immunology Laboratories

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Fall Meeting

Please stop by and say "hello" in December at this year's American Society of Hematology (ASH) Annual Meeting in New Orleans. We will be there as the Cincinnati Children's Clinical Laboratories.

THIS ISSUE'S FOCUS – Th17 Assay

Hyperimmunoglobulin E syndrome (HIES) is a rare immune deficiency typically characterized by dermatitus, recurrent infections, abnormalities of bone and connective tissue and elevated serum IgE.[1] The most common genetic mutations reported in HIES patients are in either STAT3 or DOCK8 which result in interference with the differentiation pathway of Th17 cells, causing low or non-existent cell numbers.[2]

Th17 is the name of interleukin (IL)-17 producing CD4+ T cells that are a subpopulation of T helper lymphocytes involved in immune responses to fungal and extracellular bacterial pathogens. We have developed a flow cytometric assay that detects the peripheral blood levels of Th17 cells in order to screen patients for Hyper IgE Syndrome.

In our assay, IL-17 cytokine production is stimulated by activating whole blood in the presence of Brefeldin-A to block release of intracellular cytokines, trapping cytokines produced during activation inside the cell. The activated cells are stained with surface monoclonal antibodies for phenotyping, fixed, permeabilized and stained with antibody against IL-17. The cells are then analyzed by flow cytometry. See Figure 1 and 2 for examples of the analysis on a healthy adult control as well as on a patient with HIES.

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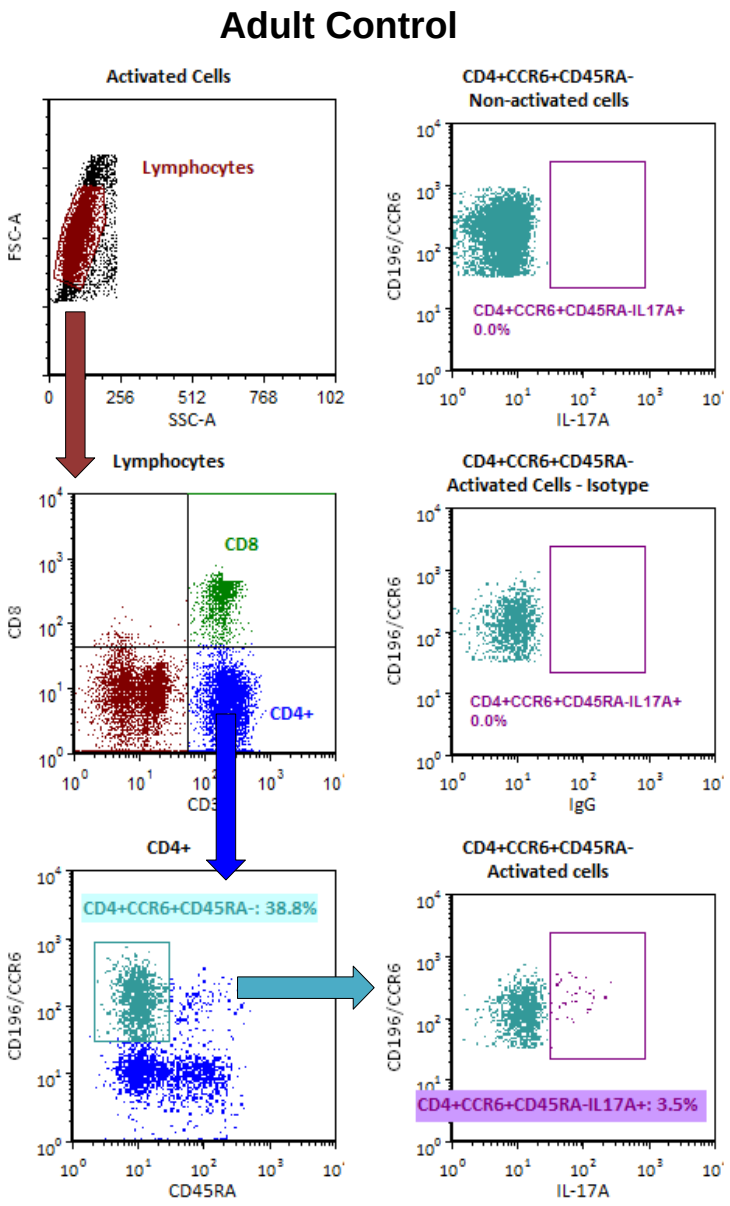

Figure 1. Adult control showing normal expression of Th17 cells. Example analysis of the Th17 assay. We first classify cells by surface phenotyping of CD4+CCR6+CD45RA- expression. We then look at the isotype staining on activated cells as well as the IL17+ staining on non-activated cells to set our IL17+ gate. 38.8% of CD4+ cells are CCR6+CD45RA-. 3.5% of CCR6+CD45RA- are IL17+.

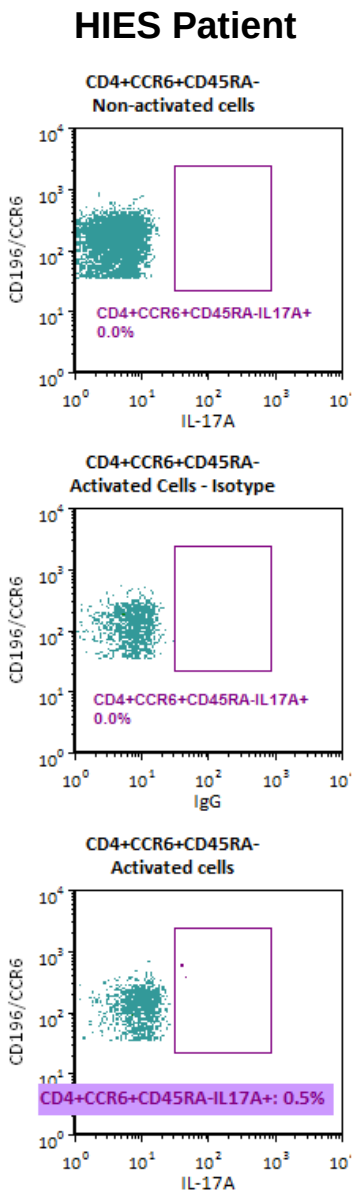

Figure 2. HIES patient showing decreased to absent IL-17 production, reflecting a lack of TH17 cells. Only 0.5% of the CD4+CCR6+CD45RA- cells are IL17+.

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Regardless of the % of CD4+ T cells which were CD45RA- CCR6+, the % of Th17 cells (that produce IL-17) was fairly constant among healthy adult controls and ranged from 0.31-1.8% of the total CD4+ T cell population (Figure 3 and Table 1).

To establish normal pediatric ranges, peripheral blood from healthy children was used to determine pediatric normal ranges of T cell populations which are CD4+ CD45RA- and CCR6+ (Table 1). This information was then used in conjunction with the adult normal range for Th17/IL17A+ cells to screen for Hyper IgE Syndrome in pediatric patients.

A limitation of the assay is our ability to run samples at various timepoints post-collection. Comparisons revealed that surface marker expression does not change following activation of samples, but that surface marker/T cell phenotype differs in samples tested immediately after blood draw versus samples which were 24 hours old. Therefore, all testing must be performed on samples rested at room temperature for 24 hours following blood draw in order to have a viable reference range.

Table 1. Shows normal ranges established for each result component as well as a result from a confirmed HIES patient for comparison.

	CCR6+CD45RA- (of CD4+)	IL17+ (of CD4+CCR6+CD45RA-)	Th17 (of CD4+)
Adults	15.6 – 47.0 %	1.2 – 4.6 %	0.31 – 1.80 %
<2 years	2.4 – 6.1 %		
2-6 years	4.2 – 12.2 %		
6-18 years	10.7 – 26.9 %		
HIES patient, 3 yo	6.8%	0.5%	0.04%

Patient Vignette:

A three-year old male was referred to the clinic for evaluation of recurrent infections and concern for Hyper IgE syndrome. His IgE was 628 (2-199 IU/mL ref range) with a history of rashes, presumed eczema, that did not respond to typical treatments, as well as respiratory infections. The Th17 assay performed in our laboratory revealed as absence of IL-17+ CD4+ CCR6+ CD45RA- cells, indicating a lack of TH17 cells (Figure 2 and Table 1). STAT3 sequencing was performed which showed a novel missense mutation (heterozygous A>G nucleotide substition) and confirmed the diagnosis of HIES. Treatment continues to be prophylactic oral antibiotics as well as topical antibacterials and topical steroids.

References

1. Paulson ML, Freeman AF, Holland SM. Hyper IgE syndrome: an update on clinical aspects and the role of signal transducer and activator of transcription 3. Curr Opin Allergy Clin Immunol 2008; 8:527.
2. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007; 357:1608.

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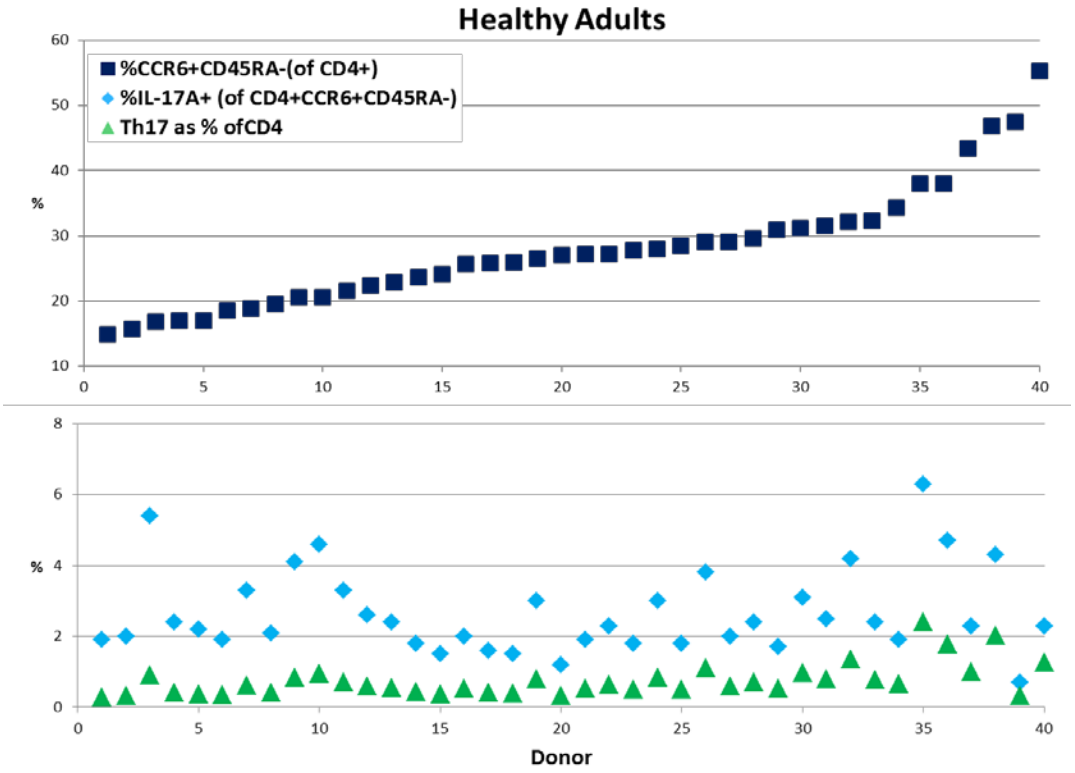

Figure 3. Results from 40 healthy adult donors.

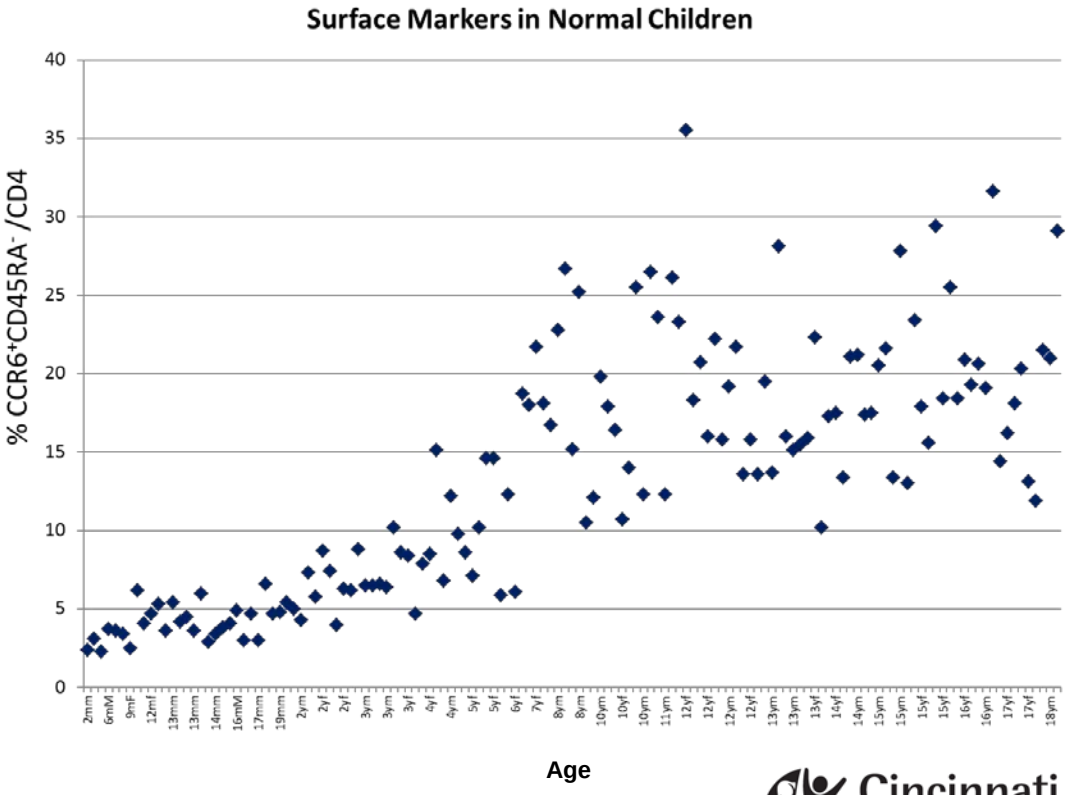

Figure 4. Surface Marker Expression in healthy pediatric donors

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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:
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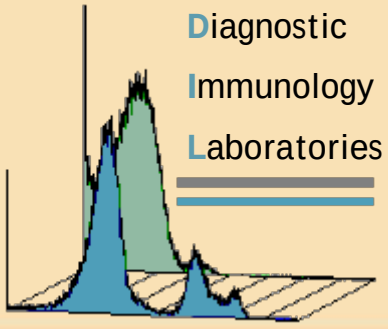

New Tests *Now* Available:

- **Th17**

Tests Coming Soon:

- ALPS Biomarkers: soluble FAS-ligand (sFASL), IL-10, and IL-18

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

"Hat Day" in the DIL during the 2013 National Medical Laboratory Professionals Week.

(Clockwise left to right, Pat Adkins, Joyce Collett, Barb Wanstrath, Victor Lafay, Carrie Gifford and Kathryn Quinn.)

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NEW Fillable Requisition on the Website:

GENERAL IMMUNOPHENOTYPING AND FUNCTIONAL TESTING

T Cell Immunophenotyping:
- ☐ Lymphocyte Subsets
- ☐ CD45 RA/RO
- ☐ TCR V beta Repertoire
- ☐ TCR alpha/beta/gamma/delta
- ☐ Lymphocyte Activation Markers
- ☐ iNKT cell enumeration

T cell Functional Studies :
- ☐ Mitogen Stimulation
- ☐ Antigen Stimulation
- ☐ CD40L/ICOS
- ☐ CTL Function
- ☐ Cytokines, Intracellular Panel
- ☐ pSTAT5

B Cell Immunophenotyping:
- ☐ Lymphocyte Subsets
- ☐ B cell panel

NK cell Functional Studies:
- ☐ NK cell Function
- ☐ CD107a (Note: cannot be accepted on Fridays)

Cytokines & Plasma Proteins
- ☐ BAFF
- ☐ Cytokine Panel: Plasma or CSF
- ☐ Neopterin: Plasma or CSF
- ☐ Soluble CD163
- ☐ Soluble IL2 Receptor/s CD25 CD25 Cytokine Panel: IL-1B,2,4,5,6,8,10 IGN-g,TNF-a, and GM-CSF

DISEASE TARGETED TESTS

Disorders of Lymphoproliferation and Autoimmunity:

Autoimmune Lymphoproliferative Syndrome (ALPS)
- ☐ ALPS Panel
- ☐ Apoptosis (Fas-Mediated)

Note: Sample must be <24 hrs old and received Thursday AM

Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked Syndrome (IPEX)
- ☐ Foxp3

Hemophagocytic Disorders:

Hemophagocytic Lymphohistiocytosis (HLH)
X-linked Lymphoproliferative Diseases (XLP)
- ☐ NK function
- ☐ CTL function
- ☐ Perforin/Granzyme B
- ☐ Soluble IL2 Receptor/sCD25
- ☐ CD107a (Note: cannot be accepted on Fridays)
- ☐ Soluble CD163
- ☐ SAP/XLP1 (if male patient)
- ☐ XIAP/XLP2 (if male patient)
- ☐ Neopterin
- ☐ iNKT

Immune Reconstitution Studies:
- ☐ Lymphocyte Subsets
- ☐ Mitogen Stimulation
- ☐ NK Function
- ☐ B Cell Panel
- ☐ CD45 RARO
- ☐ Foxp3 (T-reg enumeration)

Hyper IgE Syndrome:
- ☐ Th17 Enumeration

Combined Immune Deficiencies:
- *X-linked SCID* ☐ CD127/132 ☐ pSTAT5
- *Jak3 deficient SCID* ☐ pSTAT5
- *IL-7R deficient SCID* ☐ CD127/132
- *MHC Class I or II deficiency* ☐ MHC Class I & II
- *Zap70 deficient SCID* ☐ Zap70

Primarily Humoral Disorders:

Hyper IgM Syndrome (HIGM)
- ☐ CD40L/ICOS

Combined Variable Immune Deficiency (CVID)
- ☐ B Cell Panel
- ☐ CD40L/ICOS
- ☐ BAFF

Other Well-Defined Disorders:

Wiskott-Aldrich Syndrome (WAS)
- ☐ WASP
- ☐ WASP Post-Transplant Monitor Panel

Paroxysmal Nocturnal Hemoglobinuria (PNH)
- ☐ PNH Screen (CD59)

Neutrophil Disorders:

Chronic Granulomatous Disease (CGD)
- ☐ Oxidative Burst (DHR)

Leukocyte Adhesion Deficiency (LAD)
- ☐ Adhesion Markers (CD18/CD11b)

Other Testing and Services:
- ☐ Lymphocyte Activation Markers (Basiliximab monitor)
- ☐ CD52 (Alemtuzumab monitor)
- ☐ CD64 (upregulation on neutrophils)
- ☐ EBV Immortalized Line
- ☐ Other:_____

IN THE UPCOMING ISSUE:

- **ALPS Biomarkers**

jmcn Jeffrey Modell Centers Network

FOCIS Federation of Clinical Immunology Societies www.focisnet.org

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