Cincinnati Children's

From the Diagnostic Immunology Laboratories

Clinical Immunology Laboratory | Research Immunology Laboratory

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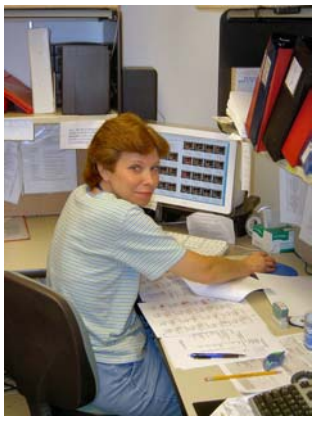
IN THIS ISSUE



Kathryn Quinn

Cincinnati Children's Hospital Medical Center, Division of Hematology/Oncology, CHRF 1301
3333 Burnet Avenue
Cincinnati, Ohio 45229
513-636-4685

This Newsletter is published semi-annually by the Staff of the Diagnostic Immunology Laboratories, Division of Hematology/Oncology at Cincinnati Children's Hospital Medical Center.

Director
Alexandra H. Filipovich, MD

Associate Director and Editor
Jack J.H. Bleesing, MD, PhD

Laboratory Manager
Darryl Hake

Patient Vignette (part 1 — Presentation)

The Clinical Immunology and Research Immunology Laboratories are integrated in a comprehensive Immunodeficiencies and Histiocytosis Program at Cincinnati Children's Hospital Medical Center (CCHMC). It interfaces with the Molecular Genetics Laboratory (http://www.cincinnatichildrens.org/research/div/genetics), as part of the Diagnostic Center for Heritable Immunodeficiencies, as well as with the Blood and Marrow Transplantation Program. To illustrate these interactions, each Newsletter will present a Patient Vignette, starting with an interesting result obtained in the Laboratory.

A Confusing Case of Class Switching?

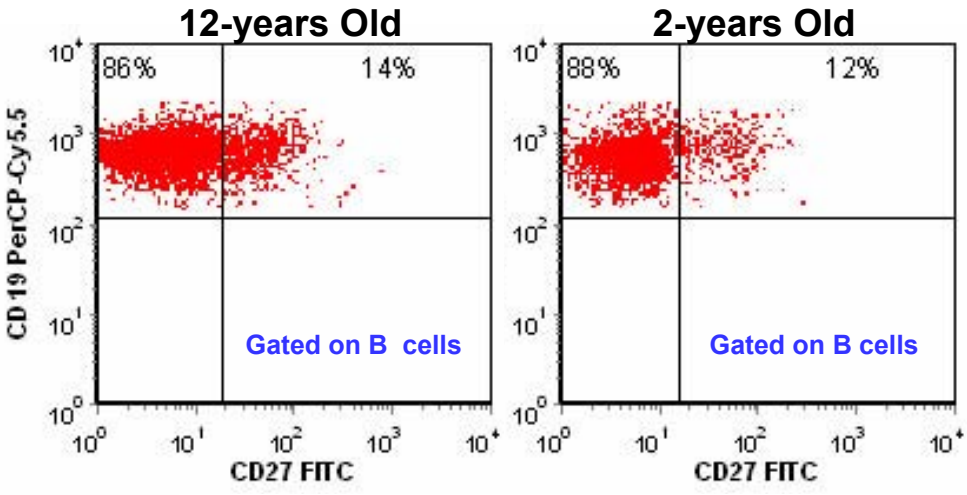
Two brothers were seen in our Immunodeficiency Clinic for an evaluation of possible X-linked Hyper-IgM (XHIGM; a.k.a. HIGM1) syndrome (see side-bar on page 8 for HIGM classification).

The oldest brother (12 at the time of evaluation) was suspected of having HIGM syndrome based on his clinical phenotype – recurrent sinus and ear infections – and the presence of high IgM levels and reduced to absent IgG, IgA, and IgE. A previously performed CD40L assay (a screening assay for HIGM1) was reportedly abnormal. The younger brother (2 yrs old) was diagnosed shortly after birth, based on a similar pattern of increased IgM reduced IgG, IgA and IgE. Both patients receive IVIG and bactrim for PCP prophylaxis.

While the CD40L assay was in progress, we reviewed their B-cell panels. The figures, shown below, represent the percentage of CD27+ B cells (on gated B-cells). In the older brother, 14% of B cells expressed CD27, while the younger brother showed a percentage of 12% CD27+ B cells.

As discussed in our **Spring-2006 Newsletter**, CD27+ B cells are measured as part of the comprehensive B-cell panel, to determine the percentage of memory B cells in peripheral blood. As shown in the figure on page 7, the percentage of memory B cells in peripheral blood is age-dependent, and typically constitutes >10% of B cells. It can be as high as 25-35% of B cells, even at a young age (and independent of prior vaccinations).

CD27-positive B cells in Hyper IgM Syndrome



See Part 2— Discussion on pages 7 and 8

Cincinnati Children's

From the Diagnostic Immunology Laboratories

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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.cincinnatichildrens.org/immunodeficiencies

New Assay Development

Phospho-STAT5 (pSTAT5) ASSAY

Introduction:

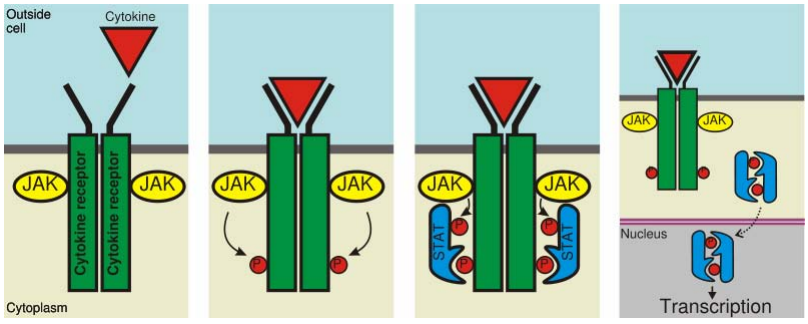
Protein phosphorylation is an important pathway for regulation of protein function in living cells. It is relevant for the process of signal transduction that regulates gene expression, cellular proliferation and differentiation. Cells respond to the environment in a stepwise mechanism. Upon engagement of a receptor (e.g., growth-, hormone-, cytokine-receptor), molecules are recruited to the receptor to relay the signal to the interior of the cell, resulting in a series of events in response to the outside stimulus. The predominant mechanism by which signal transduction occurs is through tyrosine phosphorylation of intracellular proteins, which in turn induces conformational changes in the proteins, allowing other proteins and molecules to dock and interact with the phosphorylated protein. In the case of signal transduction pathways, phosphorylation is often a transient, reversible, process, indicative of the (sequential) activation of proteins within a cascade of transduction events.

Thus, measurements of phosphorylation of specific intracellular (intranuclear) proteins provides valuable information regarding the specific pathways that have been engaged, including the origin of the cellular activation as well as the kinetics of the response to the outside stimulus. It has been well recognized that defective phosphorylation is linked to a variety of pathological states, including cancer, asthma, diabetes, inflammatory and immunodeficiency disorders.

Consequently, recognition of this central importance of protein kinase dysfunction has spurred development of specific kinase inhibitors to target certain diseases (e.g. imatinib mesylate (Gleevec) as an inhibitor of the Bcr-Abl signaling protein in chronic myeloid leukemia). Thus comparison of differences in phosphorylation events between healthy and diseased cells could be used to identify aberrant intracellular behavior that underlies certain disorders, both from standpoint of diagnostics and therapeutics.

Thanks to advances in the field of flow cytometry (FCM), multi-parameter phospho-FCM is now available to characterize multiple phosphorylated molecules simultaneously at the single cell level, using cells from all relevant tissues (see references 1-4; side-bar). An increasing number of phospho-specific antibodies recognizing phospho-epitopes (e.g. phospho-tyrosine and -serine) are now available, as are their non-phosphorylated counterparts. These include proteins belonging to the mitogen-activated protein (MAP) cascades, the Janus kinase/Signal transducer and activator of transcription (Jak/Stat) cascades, and tyrosine kinase receptors. In addition to new FCM reagents, other innovative developments include improved methods for permeabilization, allowing the reagents to bind to the phosphoproteins, new approaches to visualization and analysis of data (akin those used in proteomics), and robotics platforms to facilitate high-throughput acquisition of data.

Schematic Representation of the Jak/Stat System



Kristi Smiley

Phospho-FCM/pSTAT5; further reading:

1. Schulz et al. Curr Protoc Immunol. 2007:Unit 8.17.

2. Nolan. Hematology Am Soc Hematol Educ Program. 2006;509:123.

3. Krutzik et al. Nat Methods. 2006;3:361.

4. Lee et al. J Virol. 2008;82:3702.

5. Yao et al. Proc Natl Acad Sci USA. 2006;103:1000.

6. Fleisher et al. Clin Immunol. 1999;90:425.

7. Aboudola et al. Am J Surg Pathol. 2007;31:233.

8. Martini et al. Am J Clin Pathol. 2008;129:472.

9. Zuluaga Tora et al. Br J Haematol. 2007;139:31.

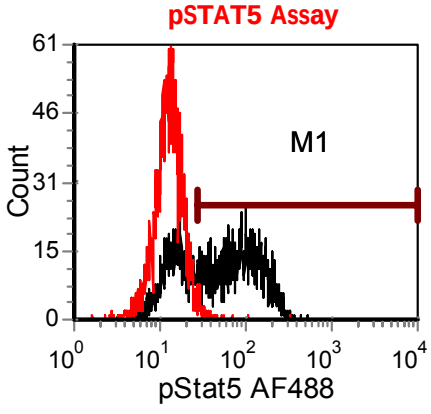
Jak/Stat Pathway:

The Jak/Stat pathways are especially relevant in the study of immunodeficiency and inflammatory disorders. A multitude of cytokines and growth factors converge in an hour-glass fashion, using limited combinations of Jaks and Stats to transduce the signals down-stream and elicit a multitude of immune/inflammatory responses. Simplified, binding of the cytokine/growth factor to the receptor triggers activation of a specific Jak, which subsequently phosphorylates tyrosine residues on the receptor (see figure on the previous page). This creates sites for interaction with matching Stat proteins that are recruited to the receptors. In turn, the Stats are tyrosine-phosphorylated by the Jaks. These phosphotyrosines then act as docking sites for other Stats, mediating their dimerization into hetero– and homo-dimers. The dimers accumulate in the cell nucleus and activate transcription of their target genes (in an hour-glass fashion).

Detection of Stat phosphorylation by phospho-FCM can test the integrity of the Jak/Stat-dependent pathways in a variety of inflammatory and immunodeficiency disorders. Some of these disorders lend themselves for relatively straightforward screening by phospho-FCM of relevant members of the Jak/Stat cascades (see reference 5). Phospho-FCM can test the transduction pathway used by the common gamma chain family of cytokines in lymphocytes. Cell surface cytokine receptors of this family include IL-2, IL-4, IL-7, IL-9, IL-15 and IL-12, while Jak3 and Stat5 represent the convergence of the signal transduction pathway.

The lethal immunodeficiency disorder; X-linked severe combined immunodeficiency (**X-SCID**) is caused by mutations in the gene (located on the X chromosome) that encodes the common gamma chain (**GC**) subunit shared by these cytokine receptors. Boys who have a mutation in this gene, lack T cells and NK cells and have defective B-cell function (despite the presence of B cells). These defects are linked to defective function of multiple cytokine receptors. In addition to X-SCID, a similar autosomal recessive form of SCID has been identified by linking the GC cytokine receptor family to abnormal cellular responses, mediated by Jak3.

Since both XSCID and Jak3-mutant SCID converge at the level of Stat5, phospho-FCM of Stat5 would provide a screening tool for these conditions. This principle is represented by the phospho-Stat5 (**pSTAT5**) assay. In this assay, whole blood is transiently stimulated by IL-2 to activate the pathway. This is followed by detection of pStat5 in selected lymphocyte populations by a phospho-specific antibody. In the figure below, an example is shown in a healthy adult. The **red line** represents pSTAT5 expression in the unstimulated condition; the **black line** represent pSTAT5 expression in CD4+ T cells following stimulation (60% positive). In addition to screening for SCID, the **pSTAT** assay can also be used to test *in vitro* B-cell function, as well as measure (constitutive) stat5 phosphorylation in other cells and conditions, such as mast cells, and malignancies. More about this assay and other phospho-FCM applications in **future Newsletters**.



Spring in Ohio

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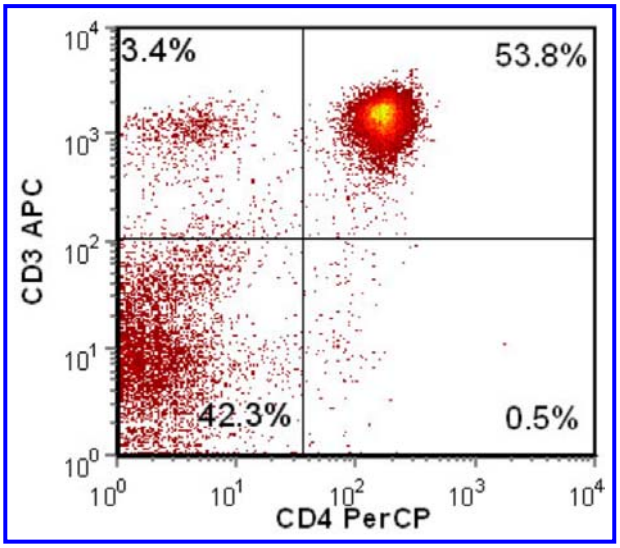
AN EXERCISE IN PATTERN RECOGNITION

The immune system depends on pattern recognition in order to detect danger signals and counteract infections. The diagnostic process of immunodeficiency disorders is largely based on pattern recognition as well. This can be relatively straightforward, such as observing absence of B cells in X-linked Agammaglobulinemia (XLA), but can also be considerably more challenging. Part of the design and execution of immunological assays at the DIL is focused on facilitating pattern recognition, and this includes looking at qualitative aspects of data, as well as evaluating data that is not part of the primary intention of the assay (i.e. the data that is not reported).

At regular intervals, we will use the Newsletter to exercise our brains in pattern recognition. Have you discovered an interesting pattern? Share it with us and have it included in a future Newsletter.

1. Skewed CD4:CD8 ratio. An ALPS panel was obtained in a patient with thrombocytopenia. The figure represents a dotplot of CD4+ T cells (right upper quadrant) versus non-CD4+ T cells (left upper quadrant), which in this case corresponds to CD8+ T cells. The CD4:CD8 ratio is about 16:1. The patient was suspected of having Wiskott-Aldrich syndrome (WAS); the WASP (WAS Protein) assay performed at the same time confirmed this diagnosis. The pattern of skewed CD4:C8 ratios (albeit not always as extreme as in this case), associated with CD8+ T-cell lymphopenia is typical for WAS. Our only recently diagnosed WAS patient, from a series of about 10 patients, who did not reveal this pattern, was an infant with acute CMV infection (also highlighting the ever-present exceptions to a rule and the need to regard data in the context of clinical info).

Skewed CD4:CD8 Ratio in WAS



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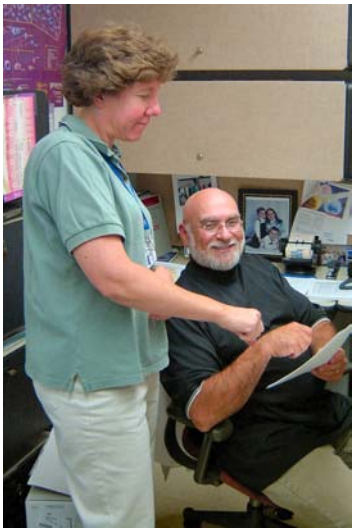
2. CD21-negative B cells. Sometimes the observed patterns suggest who ordered a particular assay. The predominant population of CD21-negative B cells (figure on next page) is a relatively common pattern, observed in patients with Systemic Lupus Erythematosus (**SLE**). This is often accompanied with other interesting patterns, including reduced CD5+ B cells, B cells with bright expression of CD27 and the presence of plasma-blasts, defined on the basis of dim CD19 expression in combination with bright expression of CD38 and/or CD138. CD21-negative B cells are also observed as part of B-cell reconstitution following B-cell depletion by rituximab, but in that scenario, the accompanying pattern includes increased CD10+/CD5+ and CD27-negative B cells and no plasma-blasts (see also **Spring-2006** and **Spring-2007 Newsletters**).

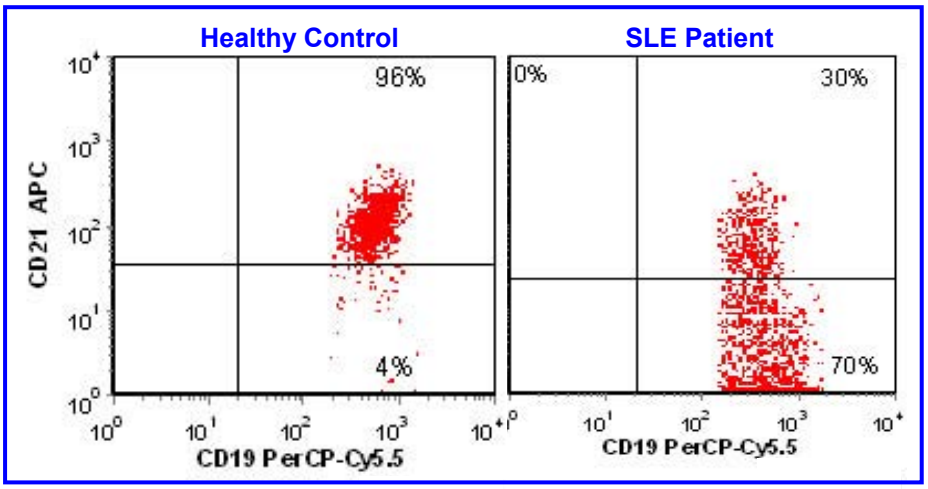
***Pattern Recognition*; markers discussed:**

- ***CD3***
- ***CD4***
- ***CD5***
- ***CD8***
- ***CD19***
- ***CD21***
- ***CD27***
- ***CD38***
- ***CD138***
- ***Gamma/delta TcR***
- ***HLA-DR***
- ***WASP***



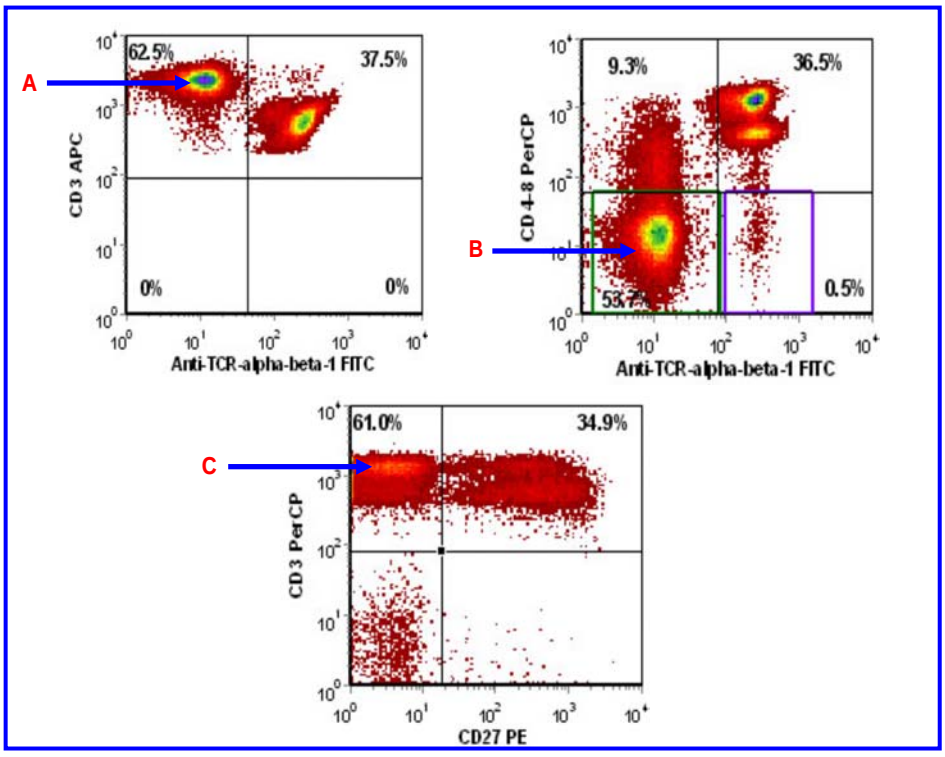
Linda Poole
Dan Marmer

CD21-negative B cells in SLE



3. Bright CD3 expression. As is demonstrated by the top left dotplot, paying attention to qualitative aspects can be revealing. It shows a proportion of T cells expressing a higher level of CD3 fluorescence intensity (**A**). These cells express the gamma/delta T-cell receptor (TcR), indirectly determined by the lack of expression of the alpha/beta TcR. As discussed in the **Fall-2007 Newsletter**, gamma/delta T cells often lack CD4 and CD8 (**B**), and may show increased or decreased expression of other markers, such as HLA-DR and CD27 (**C**).

Bright CD3 Expression on Gamma/Delta T cells



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Online Tool:

1. Go to www.genetests.org/

2. Click on GeneReviews

3. Search by Gene
- ELA2
- WAS

DIL Teamwork

ELA2/HAX1; further reading:

1. Bohn et al. Curr Opin Rheumatol. 2007;19:644.

2. Skokowa et al. Curr Opin Hematol. 2007;14:22.

3. Horwitz et al. Nat Genet. 1997;23:433.

4. Klein et al. Nat Genet. 2007;39:86.

5. Germeshausen et al. Blood. 2008 (Epub ahead of print).

6. Germeshausen et al. Blood. 2008;107:4628.

News From the Genetics Lab

New Diagnostic Tests for Congenital Neutropenia: ELA2 and HAX1

*Two new molecular diagnostic tests will join the menu of the **Diagnostic Center for Heritable Immunodeficiencies (DCHI).***

The differential diagnosis of neutropenia is extensive and includes both acquired and inherited diseases. Recent progress in unraveling the underlying genetic defects has resulted in the identification of several genetic mutations that are associated with congenital neutropenia. Congenital forms of neutropenia can be roughly classified into isolated severe congenital neutropenia (**SCN**) and neutropenia as part of congenital disorders (see reference 1; side-bar).

SCN is characterized by peripheral blood neutrophil counts that are consistently below 500/μL, and the so-called maturation arrest in the bone marrow, in which neutrophil differentiation is halted at the promyelocyte/myelocyte stage. The clinical manifestations include omphalitis after birth, recurrent skin and oropharyngeal infections, as well as deep-seated abscesses in liver, lung and subcutaneous tissues, amongst other infections. Individuals with SCN have poor wound healing and have an increased risk of myelodysplastic syndrome (**MDS**), associated with monosomy 7, and acute myeloid leukemia (**AML**). Treatment options include prompt and aggressive treatment of infections, injections with granulocyte colony-stimulating factor (**G-CSF**), periodic bone marrow studies to detect MDS, and allogeneic stem cell transplantation (**aSCT**).

A review of the literature (summarized in reference 2) suggests that 38-80% of autosomal dominant cases of SCN are linked to heterozygous **ELA2** mutations. **ELA2** encodes neutrophil elastase, a serine protease exclusively expressed in neutrophils and monocytes. How **ELA2** mutations cause SCN remains unclear. In addition to SCN, **ELA2** mutations also cause cyclic neutropenia; defined as neutropenia with counts below 200/μL for 3-5 days at ~ 3-week intervals, while peak neutrophil counts often remain below 2000/μL.

The **ELA2** mutation detection rate for cyclic neutropenia is higher than for SCN (~90-100% of cases). Genotype-phenotype relationships appear to indicate that certain mutations are predominantly associated with cyclic neutropenia, with no established risk of evolution into AML, whereas other mutations are more commonly found in SCN. It should be realized that the patterns of mutations in cyclic neutropenia and SCN are distinct when populations are studied, but can overlap when individual patients are evaluated. This suggests that distinguishing between cyclic neutropenia and SCN should be done on the basis of clinical findings and not on the basis of genotype alone.

Approximately 50 years after the first description of Kostmann syndrome, mutations in the gene encoding **HAX1** were found in the original family. Homozygous mutations in **HAX1** are associated (non-overlapping with ELA2) with autosomal recessive cases of **SCN**. **HAX1** encodes a protein critical for maintaining and stabilizing the inner mitochondrial membrane potential and protecting myeloid (progenitor) cells from undergoing apoptosis. As also observed in **ELA2** mutations, patients with **HAX1** have been shown to acquire somatic mutations in the G-CSF receptor-3 gene (**GCSF3R**), linked to the process of malignant transformation (reference 4). Genotype-phenotype relationships for **HAX1** mutations are becoming apparent. Most patients identified to date are of Middle-Eastern descent, and the vast majority carry a specific mutation (p.Trp44X). Several new mutations have recently been described, associated with the presence of an alternative splice variant (isoform-b) of **HAX1**, which is also affected by the mutations.

Other neutropenia-associated genes include **WASP** (part of the DCHI menu), while **GFI1** (and others) will join the DIL Menu in the near future.

For more information on our **DCHI**, visit us at **ASPHO** and **FOCIS**, or visit us online at: **http://www.cincinnatichildrens.org/dchi**

Patient Vignette (part 2 *continued from page 1*)

The presence of CD27+ B cells in both patients was unexpected, since HIGM1 patients typically lack memory B cells in peripheral blood (compare with figures below, obtained from a 8 year old **HIGM1** patient, and four controls with increasing age [CD27 on Y-axis]). The results of the CD40L assay provided the context for these results, as both patients showed normal baseline CD40L expression, as well as normal upregulation of CD40L expression following T-cell activation. The CD40L gene was sequenced in both patients to verify the discrepancy between these and previously obtained results. No mutations in the gene encoding CD40L were identified.

In light of the clinical phenotype and immunological phenotype – our workup showed normal to slightly increased IgM levels and near-absent IgA and IgE levels, other forms of HIGM were considered. Given the apparent X-linked inheritance pattern, mutations in the gene encoding **NEMO** were ruled out, while normal CD40 expression (also part of the B-cell panel) ruled out **HIGM3**. Subsequent genetic testing of the gene encoding Activation-Induced Cytidine Deaminase (AICDA) revealed bi-allelic mutations, consistent with a diagnosis of **HIGM2**, while the gene encoding uracil-N-glycosylase (UNG, **HIGM5**) was normal.

HIGM Syndrome Classification:

- HIGM1: defects in CD40L gene (X-linked)

- HIGM2: defects in AICDA gene

- HIGM3: defects in CD40 gene

- HIGM4: unknown defect(s)

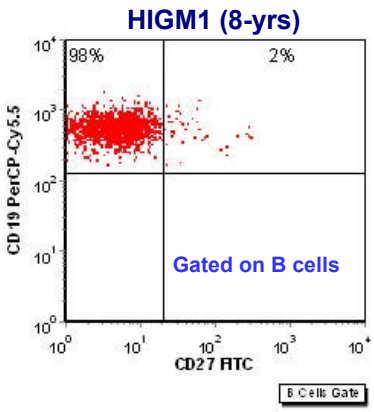
- HIGM5: defects in UNG gene

- HIGM6: defects in NEMO gene (X-linked)

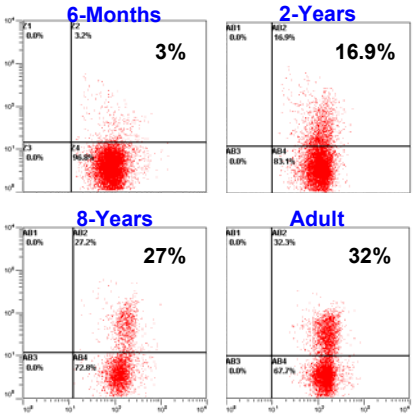
2%

Gated on B cells

Lack of CD27-positive B cells in (Classic) HIGM1



Age-dependent Increase in CD27+ B cells



Hyper-IgM syndromes are due to so-called B-cell intrinsic immunoglobulin class switch recombination (Ig-CSR) deficiencies (see reference 1 in side-bar). CSR is important during the germinal center reaction to improve the response to infections, through isotype-switching from IgM to IgG, IgA, or IgE (with specialized functions, e.g. complement activation or presence in mucosal secretions). The germinal center reaction also includes somatic hypermutation (SHM), which incorporates mutations in the immunoglobulin molecule in a stochastic high-frequency manner, and increases the affinity of the molecule for its antigens.

Both CSR and SHM occur in the germinal center, but are not dependent on each other. Although it has been considered that both CSR and SHM require CD40L interaction on activated T cells with CD40 on B cells, it has been shown that CD40L-independent pathways exist. In the case of CSR, the BAFF system (see **Fall-2007 Newsletter**) can substitute for CD40L/CD40 (with appropriate cytokines and B-cell antigen receptor engagement), while SHM has been found in IgM-expressing B cells, residing in the splenic marginal zone (representing a defense mechanism against blood-borne encapsulated bacteria).

Spring in Ohio

Part 2—Continued

Going back to the issue of CD27+ B cells in HIGM syndromes; it has been proposed that peripheral blood memory B cells are derived from B cells that have (successfully) undergone SHM during the germinal center reaction. Immunophenotypically, they express CD27. While in many cases, the lack of CD27+ B cells is indicative of a defective generation of memory B cells (see figure on page 4 of the **Spring-2006 Newsletter**), the reverse – presence of CD27+ B cells indicative of the presence of memory B cells – is not the case, as demonstrated by HIGM2 patients. In the case of Ig-CSR deficiency on the basis of autosomal recessive AICDA defects, many patients show a normal percentage of CD27+ B cells; yet these cells lack acquisition of somatic hypermutations.

The situation, however, is more complex as AICDA mutations located in the C-terminal part of AICDA have been found in which only CSR is defective, but SHM is preserved. In these cases, the presence of CD27+ B cells may thus appropriately reflect the presence of memory B cells. Lastly, autosomal dominant transmission of AICDA mutations have been found in several patients with variable immunodeficiency phenotypes, and preserved SHM in some, but not all, patients. The mutations affect the nuclear export signal (NES) domain of AICDA. CD27+ B cells are present in these patients.

An important clinical clue in the evaluation of HIGM patients is the presence of lymphoid hyperplasia in certain patients with HIGM2. Pathological examination of biopsied lymph nodes revealed the presence of giant germinal centers, filled with highly proliferating B cells (also referred to as progressive transformation of germinal centers). This histopathological entity is characteristically found in ALPS and in forms of Hodgkin's lymphoma. Of note, the older brother had shown lymphadenopathy, as well as tonsillar hypertrophy in the past.

Obtaining an accurate (genetic) diagnosis has practical and prognostic implications. These include the association with autoimmunity, and liver disease (associated with Cryptosporidium infection), the risk of PCP pneumonia (and need for PCP prophylaxis), and the risk of lymphoma that are relatively well characterized in **HIGM1**, but – so far -do not appear to be present in **HIGM2**. On the other hand, HIGM2 (as wells the other HIGM forms) are somewhat "new" disorders. And thus, more needs to be learned about these, and other conditions of defective CSR and/or SHM. From a practical standpoint, CD27 measurement on B cells should be regarded with caution, and in the context of other information. Other (flow-based) methods are needed as well in order to measure memory B cells (see **future Newsletter**).

HIGM; further reading:

1. Durandy et al. Adv Immunol. 2007;94:275.

2. Notarangelo et al. J Allergy Clin Immunol. 2006;117:855.

3. Durandy et al. Immunol Rev. 2005;90:554.

4. Etzioni and Ochs. Pediatr Res. 2004;56:519.

5. Jain et al. J Clin Invest. 2004;114:1593.

6. Imai et al. J Clin Invest. 2003;112:136.

7. Bleesing and Fleisher. Cytometry B Clin Cytom. 2003;51:1.

RECAP

In this Newsletter, the following assays were discussed:

1] B cell Panel
Flow cytometry-based panel to provide an immunophenotypic overview of B-cell development and differentiation, representing the main circulating B-cell subsets. Also used to monitor B-cell reconstitution following B-cell depletion. See also **Spring-2006 Newsletter** and **Spring-2007 Newsletter**.

2] CD40L Assay
Flow cytometry-based screen for HIGM1, measuring expressing and up-regulation of CD40L on activated T cells. Assay includes measuring expression/up-regulation of ICOS to screen for ICOS defects, as well as to determine overall integrity of T-cell activation. See also Patient Vignette in **Fall-2007 Newsletter** and **Spring-2006 Newsletter** (ICOS).

3] pSTAT5 Assay
Flow cytometry-based assay to measure phosphorylation of Stat5 in vitro. It is intended to complement current screening assays for SCID. It will officially be added to the DIL Menu in the near future, but is currently available by request (contact the DIL for details).

4] ALPS panel
Assay to screen for ALPS and determine nature of DNTC populations. See also **Fall-2005 Newsletter** and **Fall-2007 Newsletter**.

5] WASP Assay
Flow cytometry-based assay to screen for Wiskott-Aldrich Syndrome (WAS). See also Patient Vignette in **Spring-2007 Newsletter**.

6] sCD163
ELISA-based assay to measure soluble CD163; a biomarker for activated macrophages and histiocytes in hemophagocytic disorders, such as HLH and macrophage activation syndrome (MAS). sCD163 correlates inversely with cell surface CD163 expression on monocytes that can be measured with the (flow-based) Leuko64 assay.

BULLETIN BOARD

Darryl's Team

FEEDBACK

We would like to hear from our Customers. We invite you to share your questions and comments with us . This can be regarding existing assays, new assays that you might be interested in, the way we report results, other services that we can provide, etc. Feel free to send/fax/email your comments to us (fax: 513-636-3546; email: immunodeficiencies@cchmc.org).

CONVENIENT ACCESS TO PATIENTS' RESULTS: LABTEST.COM

In the **Spring-2007 Newsletter**, we introduced **LABTEST.COM**

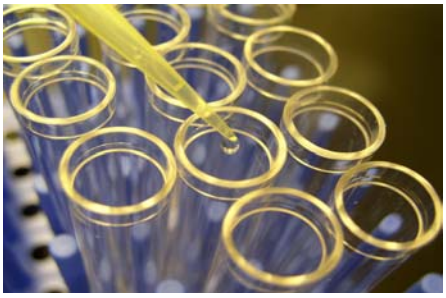
This Web-based program provides a platform for easy and fast access to patients' results. Using any standard internet browser, Labtest.com enables you to view reports from the Diagnostic Immunology Laboratories the moment they are generated. There's no need to wait for a faxed report. For more information or to establish an account, contact our customer service representative; Julie Beach, at 513-636-4685 or julie.beach@cchmc.org

THE SOLUBLE CD163 ASSAY IS BACK!

Back on the DIL Menu: the **soluble CD163 (sCD163) assay**

CD163 is a member of the cysteine-rich scavenger receptor superfamily. It is a protein that is almost exclusively expressed on human monocytes and macrophages and their derivatives. It is cleaved by a matrix metalloproteinase into a soluble form in response to pro-inflammatory stimuli. It has been determined that increased levels of soluble CD163 (**sCD163**) are specific for macrophage activation in the context of **HLH**, and not reflective of inflammation per se. In combination with soluble IL-2Ralpha levels, measurement of sCD163 is helpful in determining histiocyte activation (in parallel with measuring ferritin) in HLH and macrophage activation syndrome (**MAS**); at baseline and in response to HLH therapy (see also Bleesing et al. Arthritis Rheum. 2007;56:965).

The **sCD163** assay has been completely overhauled, using new reagents. In addition, a new age-appropriate reference range has been established. In cases of low sCD163 levels, but suspected HLH/MAS activity, cell surface expression of CD163 on monocytes can be determined, using our CD64 assay (Leuko64 kit, see **Fall-2005 Newsletter**) as a control measurement, given the inverse relationship between cell surface and soluble CD163.



COME VISIT US AT ASPHO AND FOCIS!

The DIL is a component of the **Diagnostic Center for Heritable Immunodeficiencies** (**DCHI; http://www.cincinnatichildrens.org/dchi**). The DCHI will be present at the 21st Annual Meeting of the American Society of Pediatric Hematology/Oncology (**ASPHO**), **May 14 — May 17**, in **Cincinnati**, as well as at the Annual Meeting of the Federation of Clinical Immunology Societies (**FOCIS**), **June 5 — June 9**, in **Boston**.

DIL MENU

CURRENT MENU OF AVAILABLE TESTS

DIAGNOSTIC IMMUNOLOGY LABORATORY
part of the division of Hematology/Oncology
Phone: 513-636-4685 Fax: 513-636-3861
www.cincinnatichildrens.org/immunodeficiencies

Test Requisition Form 111706

Informative newsletters are available at www.cchmc.org/immunodeficiencies

Samples must be received within 24 hours of being drawn. Send at room temperature (unless otherwise noted) and send using
FIRST OVERNIGHT PRIORITY SHIPPING*:
Julie Beach
Cincinnati Children's Hospital Medical Center
Hematology/Oncology CHRF1301
3333 Burnet Avenue, Cincinnati, OH 45229
we are now requesting that samples be sent using the FIRST OVERNIGHT option to ensure timely delivery.

Patient Name_____ **Date of Sample**_____ **Time of Sample**_____

Medical Record #_____ **Date of Birth** _____ Sex: ❑ Male ❑ Female

Race: ❑African American ❑American Indian ❑Asian ❑Hispanic ❑White ❑Other (specify):_____

Diagnosis or reason for testing_____ ICD9 code_____

Medications: _____ Has the patient undergone BMT? ❑ no ❑ yes date of BMT_____

Test(s) being requested: ***Note: Results of a same day CBC/Diff must accompany the sample where indicated.***
Shading indicates those tests that MUST also have a Lymphocyte subset analysis ordered

❑ ALPS panel	***CBC/Diff required**	CPT #88185x9	$424	3ml (1ml) EDTA
❑ Antigen Stimulation		CPT #86353	$467	10ml (5ml) Sodium Heparin
❑ B Cell Panel	***CBC/Diff required**	call for CPT codes	$350	3ml (1ml) EDTA
❑ CD40L (CD154)		CPT #88184; 88185x3	$164	5ml (3ml) Sodium Heparin
❑ CD45RA / CD45RO		CPT #88184; 88185x4	$409	3ml (1ml) EDTA
❑ CD64 (Leuko64)		CPT #88184	$51	1ml (0.5ml) EDTA
❑ CD132 / CD127	***CBC/Diff required**	CPT #88184; 88185x5	$493	3ml (1ml) EDTA
❑ CTL function		CPT #86849	$496	10ml (5ml) Sodium Heparin
❑ Cytokines, Intracellular (IL-4, IFN-γ, TNF-α)		CPT #88184; 88185x2	$958	3ml (2ml) Sodium Heparin
❑ Cytokines, Plasma (panel of 10)		CPT#83520	$1460	5ml (3ml) EDTA
❑ EBV immortalized cell line		CPT #86849	$181	3ml Sodium Heparin
❑ Check here if this is for research purposes only; signed consent must accompany sample				
❑ EDN (Eosinophil-derived neurotoxin)		CPT #83520	$719	3ml (1ml) EDTA
❑ Eotaxin-3		CPT #83520	$445	3ml (1ml) EDTA
❑ Foxp3	***CBC/Diff required**	CPT #88184	$412	3ml (1ml) EDTA
❑ Lymphocyte Activation Markers		CPT #88184; 88185x8	$588	5ml (2ml) Sodium Heparin
❑ Lymphocyte subsets	***CBC/Diff required**	call for CPT codes	$318	3ml (1ml) EDTA
❑ Mitogen Stimulation		CPT #86353	$702	10ml (5ml) Sodium Heparin
❑ Neutrophil function tests (specify from the list below) **must be scheduled in advance**				**not available to non-local customers
❑ Oxidative Burst ❑ Adhesion Markers ❑ **Phagocytosis & Killing ❑ **Chemotaxis				
❑ NK function		CPT #86849	$397	10ml (5ml) Sodium Heparin
❑ Perforin/Granzyme B		CPT #88184	$427	3ml (1ml) EDTA
❑ PNH Screen (CD59)		CPT #88184	$89	3ml (1ml) EDTA *ON ICE*
❑ SAP protein (for R/O XLP)		CPT #88184	$433	3ml (1ml) Sodium Heparin
❑ Soluble IL-2Rα		CPT #83520	$220	3ml (1ml) EDTA or Na Hep
❑ TCR α/β / TCR γ/δ		CPT #88184; 88185x4	$477	3ml (1ml) EDTA
❑ TCR V beta clonogram		CPT #88184; 88185x23	$766	3ml (2ml) EDTA
❑ WASP	***CBC/Diff required**	CPT #88184	$200	5ml (3ml) Sodium Heparin
❑ Other _____				

Diagnostic Immunology Laboratories

FOCIS Federation of Clinical Immunology Societies www.focisnet.org

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