Cincinnati Children's

From the Diagnostic Immunology Laboratories

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Carol Moore

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.

An 11-year-old boy presented with recurrent deep skin ulcers, which first began at the age of three years. The ulcers were similar in appearance to pyoderma gangrenosum, an uncommon form of skin ulceration, seen in a variety of autoimmune disorders, such as inflammatory bowel disease, arthritis, vasculitis and systemic lupus erythematosus. The ulcers involved many body areas, including the scalp, trunk, arms and legs, extending deep into the underlying subcutaneous tissues. They typically were very painful, usually sterile, and required weeks to months to heal. The scars displayed an unusual atrophic appearance that resembled "cigarette paper". Over the years, the patient has – unsuccessfully – undergone repeated evaluations in an attempt to diagnose an underlying disorder.

He has been treated with various medications, commonly used to treat pyoderma gangrenosum, such as corticosteroids, TNG-antagonists, cyclosporine, among others.

More recent skin ulcers have been complicated by both bacterial and fungal superinfections. Of note: the hospital course until that moment had been free of significant infections (both related to his skin manifestations, as well as in other places). During episodes of skin ulceration, he would develop highly elevated white blood cell counts, typically exceeding 50,000/mm3, with most of the cells being neutrophils ("leukemoid reactions"). On the basis of these characteristics, and the new infectious complications, additional laboratory testing was obtained.

Reduced Expression of CD11b/CD18 in Patient with Pyoderma Gangrenosum

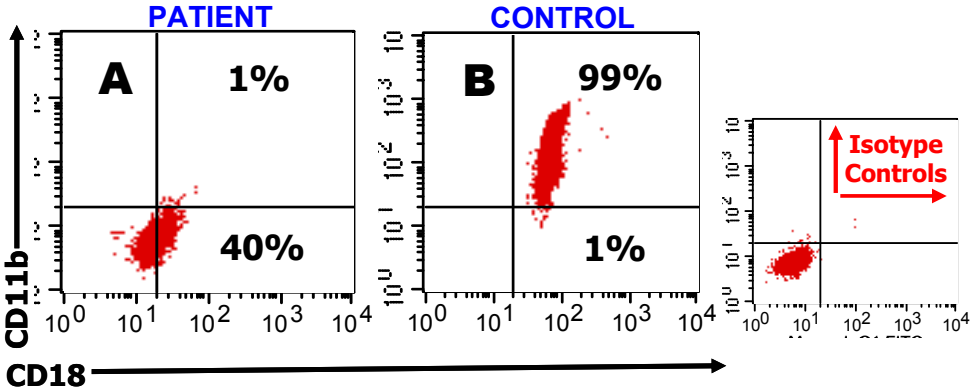

Figure 1: Adhesion Markers Assay in patient (left) and healthy control (middle).

The **figure** represents the results of the flow cytometric assay "Adhesion Markers", which is a component of the "Neutrophil Function Tests". The principle of the assay is the combined detection of the adhesion molecules CD11b and CD18 (members of the β2 integrin family) on the cell surface of neutrophils (and other white blood cells). The assay showed a marked reduction of CD11b/CD18 (dual-parameter **dotplot** on the **right**), as compared to the healthy control (**middle dotplot**; **isotype controls** on the **right**). The assay was repeated following neutrophil activation using several stimuli, to determine up-regulation of CD11b/CD18. No up-regulation was observed.

See Part 2– Discussion on page 9

Patient Vignette contributed by Claas Hinze, MD and Rebecca Marsh, MD

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

Monitoring B-cell Depletion Therapy

BAFFLED ABOUT B-CELL RECOVERY?

In the previous Newsletter, a relatively well-recognized phenomenon of B-cell recovery following rituximab was shown and discussed: widely variable kinetics of B-cell reconstitution, with B-cell counts reaching or exceeding pre-rituximab levels after only a few months in some individuals, while showing no substantial recovery in others. It was suggested that this might be related to the BAFF system.

The BAFF system (see sidebar on page 3 for an acronym legend) is a critical component in B-cell homeostasis. The seemingly unremitting publication of review articles, editorials and commentaries is indicative of the importance of this system, but also of the steep angle of the BAFF-learning curve. Before going into more detail, it is important to remember that many attributes and characteristics of the BAFF system are based on studies in mice, including mice with genetically altered BAFF components. The BAFF system belongs to the tumor necrosis factor (TNF) superfamily, and can be roughly divided into secreted factors/ligands and receptors. On the ligand side, the main players are: homotrimeric or polymeric BAFF (proteolytically cleaved from the cell surface membrane), APRIL (only secreted form), and BAFF-APRIL (in heterotrimeric combinations). Focusing on BAFF, a variety of hematopoietic and non-hematopoietic cell types produce/secrete BAFF, but – importantly – normal B cells do not. On the receptor side are TACI, BCMA and BAFF-R. According to the literature, all 3 receptors are expressed on B cells, although our own data in humans, including children, suggest that this is not the case (work in progress). The literature also indicates that BCMA is expressed on plasma cells, and TACI on macrophages and T-cell subsets. BAFF (and perhaps a truncated form of APRIL) bind to BAFF-R, while both BAFF and APRIL engage the other receptors.

Peaks and Valleys of BAFF Levels and B-cell Counts

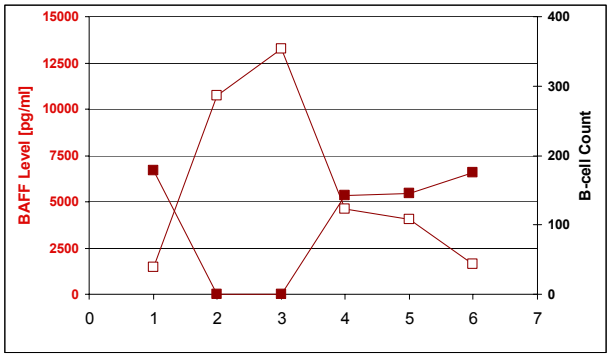

Figure 2a: Example of B-cell reconstitution kinetics, showing time period post-rituximab on X-axis, BAFF levels on left Y-axis (open squares) and B-cell counts on right Y-axis (closed squares).

BAFF-R and BCMA function as positive regulators of BAFF-induced effects, while TACI is characterized as a negative regulator. The main physiologic effect of BAFF is to act as a survival factor for B cells. B cells are not uniformly receptive to respond to BAFF-induced signaling. It appears that recent bone marrow emigrants (immature B cells) that are in a transitional phase to become mature (naïve) B cells are most responsive to BAFF. Subsequent stages of B-cell differentiation are less responsive to BAFF, while sensitivity to BAFF-induced survival returns at the plasma cell stage. Concordant with other members of the TNF superfamily, BAFF production/secretion is subject to complex regulatory mechanisms that likely includes a feedback system sensing the overall status of the B-cell compartment (e.g. number of circulating B cells and/or number of tissue-resident B cells).

Thus, in a simplistic view, removal of B cells (e.g. through rituximab) signals the BAFF-producing machinery to increase BAFF secretion, which, in combination with other growth/survival factors, drives repopulation of the B-cell compartment.

Figure 2a (previous page) shows kinetics of B-cell recovery in relation to BAFF levels post rituximab that would fit this view. In this individual (who received multiple doses of rituximab for complex autoimmune cytopenias), one can recognize an almost mirror image between B-cell counts (closed squares) and BAFF levels (open squares). Going back to the figure of varying B-cell recovery kinetics, shown in the previous Newsletter, one can envision that BAFF levels show similar variability (**figure 2b**, below).

BAFF SYSTEM ACRONYM LEGEND

BAFF

B-cell Activation Factor from the Tumor Necrosis Factor Family

APRIL

A Proliferation-Inducing Ligand

BCMA

B-cell Maturation Antigen

TACI

Transmembrane Activator and Calcium Modulator and Cyclophilin Ligand Interactor

Varying BAFF Levels Post Rituximab

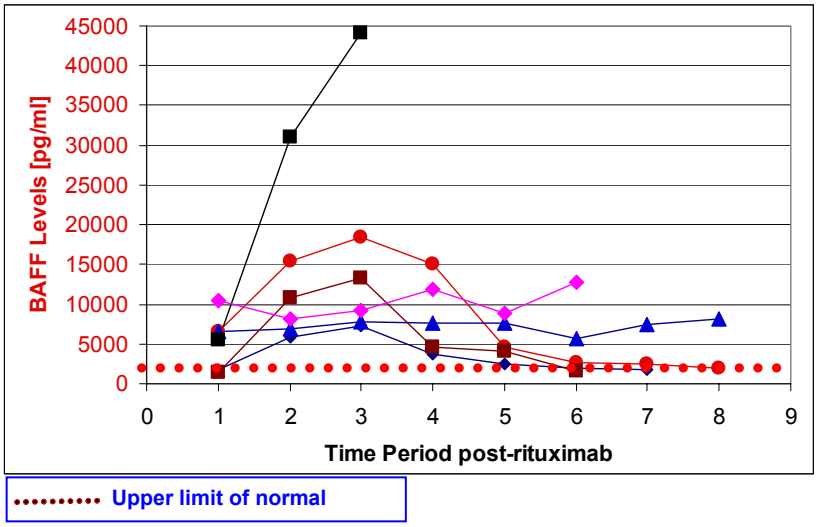

Figure 2b: Varying BAFF results in 5 individuals post-rituximab

Our preliminary experience suggests that while there often are reciprocal relationships between B-cell counts and BAFF levels, this is not a set-in-stone rule. Several examples are shown to illustrate this further. In **figure 2c** (next page), two individuals are shown with different kinetics. The individual represented by the black squares received multiple doses of rituximab for autoimmune cytopenias on a background of DiGeorge syndrome. B-cell counts dropped precipitously, but have shown a quick and brisk recovery, in the context of sharply rising BAFF levels (that are still going up). The other individual received rituximab for an EBV-driven hemophagocytic lymphohistiocytic (HLH) disorder. In this case, B-cell counts exceeded baseline levels within months after multiple doses of rituximab, concurrently with only a modest increase in BAFF levels. This despite the fact that, as has been suggested in the literature, EBV-infected B cells can violate the "rule" that (normal) B cells do not produce BAFF (i.e. produce their own survival factor).

As is clear from **figures 2b** and **2c**, BAFF levels were already elevated at baseline. In fact, since the inception of measuring BAFF levels, it has been our experience that BAFF levels are often increased at baseline (especially in autoimmune cytopenias). This has been the experience in many studies in humans regarding BAFF levels in immunologic disorders (as nicely tabulated by Mackay et al, 2007 – see sidebar on page 4).

DIL Downtime

Different Profiles of BAFF and B-cell Recovery

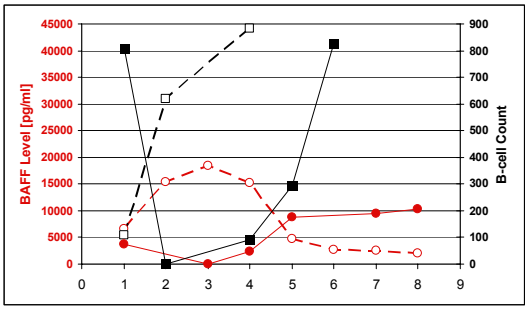

Figure 2c: Reconstitution kinetics, showing time period post-rituximab on X-axis, BAFF levels on left Y-axis (open squares and circles) and B-cell counts on right Y-axis (closed squares and circles).

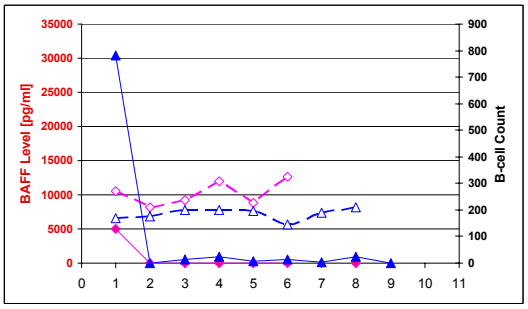

Figure 2d: Reconstitution kinetics, with time period post-rituximab on X-axis, BAFF levels on left Y-axis (open triangles and diamonds) and B-cell counts on right Y-axis (closed triangles and diamonds).

The next two individuals (**figure 2d**, above), also show elevated pre-rituximab BAFF levels. In contrast to the previous examples, no significant further increase in levels was observed, despite the fact that B-cell depletion was complete and ongoing. Both subjects were treated for chronic ITP. Of note: these profiles of B-cell depletion without a subsequent rise in BAFF levels would suggest that the explanation, referenced in the literature, that BAFF levels sharply increase following rituximab, because of a sudden depletion of BAFF receptors, is not correct (or at least not complete).

It has also been suggested that high baseline BAFF levels would provide a set-up for rapid B-cell reconstitution and relapse of the autoimmune disorder. Our experience so far indicates that this is not the case: B-cell counts have not returned to normal in these and several other individuals with similar high baseline BAFF levels. In addition, no relapse of pre-rituximab conditions has been observed regardless of the speed of B-cell reconstitution and regardless of BAFF kinetics. How BAFF levels should be interpreted in combination with B-cell counts and B-cell phenotype remains far from straightforward. The conclusion that most safely can be drawn at this point is that many BAFF measurements, in combination with B-cell counts and other relevant measurements in large numbers of individuals with different disorders over a prolonged period of time are needed to become "un-baffled" by the BAFF system.

With every biomarker, one should consider the fact that variations in levels might be reflective of genetic variability that has the potential to influence the biology of the marker. Whether this also applies to BAFF or other constituents of the BAFF system will be a topic for a future discussion on genetic controllers of B-cell reconstitution.

BAFF*; *further reading:

1. Mackay et al. Curr. Opin. Immunol. 2007;19:327.

2. Cartron et al. Blood. 2004;104:2635.

3. Lavie et al. Ann. Rheum. Dis. 2007;66:790.

4. Vallerskog et al. Arthritis Res. Ther. 2006;8:R167.

5. Seror et al. Ann. Rheum. Dis. 2007;66:351.

Fall in Ohio

Down the Pipeline*

CD107a: TURNING CYTOTOXICITY INSIDE-OUT

In the last Newsletter, a flow cytometric (FCM) assay to measure cytotoxicity was introduced (granzyme B transfer assay). This assay is based on transfer of granzyme B from effector cell to target cell. Because of the multi-parameter capabilities of the FCM platform, the assay lends itself to measure more than just killing of target cells.

Measuring NK-cell function is an integral component in the evaluation of hemophagocytic disorders (e.g. HLH). Insight into the cytotoxic machinery can guide the diagnostic workup and can help in formulating the search for genetic disorders. To this effect, functional assays are commonly combined with the perforin/granzyme B assay.

Research and Development are integral parts of the Clinical Immunology and Research Immunology Laboratories at CCHMC, and are conducted in close collaboration with the Immunodeficiencies & Histiocytosis Program.

Lisa Filipovich

Originally developed to screen for genetic defects in the gene encoding **perforin** (**PRF1**), the combination of measuring intracellular perforin as well as intracellular granzyme B has provided additional profiles that appear characteristic of HLH scenarios. Thus, reduced presence of perforin in NK-cells and T-cell subsets is characteristic of perforin mutations, while increased expression of perforin and granzyme B is observed in other forms of HLH including primary inherited forms due to mutations in the gene encoding **MUNC13-4**, as well as secondary causes. To distinguish between these different scenarios, in the next phase of the cytotoxicity assay makeover, the screening assay will be expanded to link perforin/granzyme B profiles more directly to MUNC13-4 mutations. It is anticipated that after a period of testing and evaluation, phenotypic and functional assays of cytotoxicity will be consolidated into a single platform (FCM) method.

Mutations in MUNC13-4 account for approximately 25% of familial forms of HLH (compare with ~35% perforin for mutations).

MUNC13-4 is involved in priming of the secretory lysosomes (referred to as lytic granules in cytotoxic lymphocytes, such as NK cells). Specifically, it facilitates exocytosis of these granules (containing perforin and granzyme B), such that they can engage and interact with the target cells. During this process of exocytosis, the granules fuse with the membrane of the cytotoxic cell. This feature has been exploited in an assay that measures mobilization (incorporation) of the lysosome-associated membrane-protein 1 (LAMP-1; also known as CD107a) into the cell membrane.

The basic principle of the assay involves incubating NK cells with the appropriate target cells (e.g. K562 cells) for a defined period of time and measuring CD107a expression on the surface of NK cells. Up-regulation (mobilization) of CD107a is then compared to a baseline (un-stimulated) status. Due to the fact that the process of granule exocytosis does not occur in the context of defective MUNC13-4, measuring CD107a mobilization would be a useful assay to screen for this familial form of HLH.

CD107a Mobilization Assay to Screen for MUNC13-4 Mutations

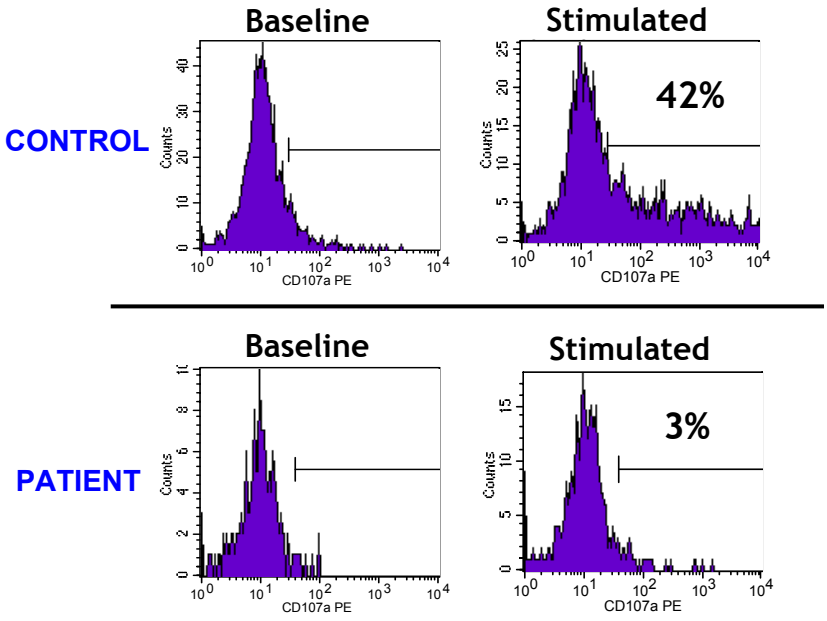

Figure 3: CD107a expression. Top histograms show up-regulated CD107a expression following incubation with K562 cells (compared to baseline) in a healthy control; the bottom histograms reflect a patient with a lack of CD107a mobilization and bi-allelic MUNC13-4 mutations.

CD107a; further reading:

1. Marcenaro et al. Blood. 2006;108:2216.

2. Alter et al. J. Immunol Methods. 2004;294:15.

3. Betts et al. Methods. Cell. Biol. 2004;75:495.

4. Penack et al. Int. J. Hematol. 2007;85:326.

LAB PERSONNEL

Darryl Hake
Julie Wiltse
Debbie Fearing
Barbara Lawall
Virgil Villanueva
Terri Ellerhorst
Sue Vergamini
Linda Poole
Maureen Powderly
Susan Lee
Patricia Adkins
Joyce Villanueva
Leslie Klinker
Kathryn Quinn
Carol Moore
Yan Zhong
Daniel Marmer
Julie Beach
Carrie Koenig
Barbara Wanstrath
Kristi Smiley
Lindsay Dunn
Holly Allen

Figure 3 (previous page) shows four histograms to illustrate the assay. The histograms reflect CD107a expression on NK-cells, as determined by CD56 expression and lack of CD3 expression. The perforin/granzyme B assay in this patient revealed up-regulated expression of perforin and granzyme B in both NK cells, as well as CD8-positive T cells, while NK-cell function was decreased. Genetic analysis showed bi-allelic mutations in **MUNC13-4**.

Due to the fact that this assay is in essence a NK-cell function test, measuring CD107a mobilization, can be combined with the granzyme B transfer assay. Similar to other screening assays, it is anticipated that interesting patterns of CD107a expression will become apparent in a variety of clinical scenarios beyond its original intend as a screening tool for MUNC13-4 mutations. Once combined with the Granzyme B transfer assay, these profiles may point to novel defects in cytotoxicity, and perhaps clear a pathway to the discovery of other genetic defects in HLH (keeping in mind that a large proportion of cases of familial HLH currently lack a genetic diagnosis).

These observations may invite new questions and perhaps another round of Cytotoxicity Makeover in the future.

Immunophenotyping 101

NEITHER CD4 NOR CD8: DOUBLE-NEGATIVE T CELLS

In the late 1970s, the dichotomy between T-lymphocyte subsets, based on CD4 and CD8 expression was firmly established. It seems that no other immunophenotypic marker that is exclusively expressed on CD4+ or CD8+ T cells has been found since (the Reader is kindly invited to submit references indicating otherwise). In the traditional dual-parameter dotplot; CD4 and CD8 are shown on alternate (**X** or **Y**) axes, corresponding to two different fluorescence (FL) channels (see left dotplot in **figure 4a**). As can be seen in this example, T cells are predominantly CD4+ (lower right [**LR**] quadrant), or CD8+ (upper left [**UL**] quadrant). In peripheral blood, few T cells express both CD4 and CD8 (**UR** quadrant).

In this edition of Immuno-101, the focus will be on the **LL** quadrant: T cells that express neither CD4 nor CD8. These cells are referred to as double-negative T cells (DNTCs). These cells can be anything from a curiosity to a diagnostic finding.

Double-Negative T cells Express Neither CD4 Nor CD8

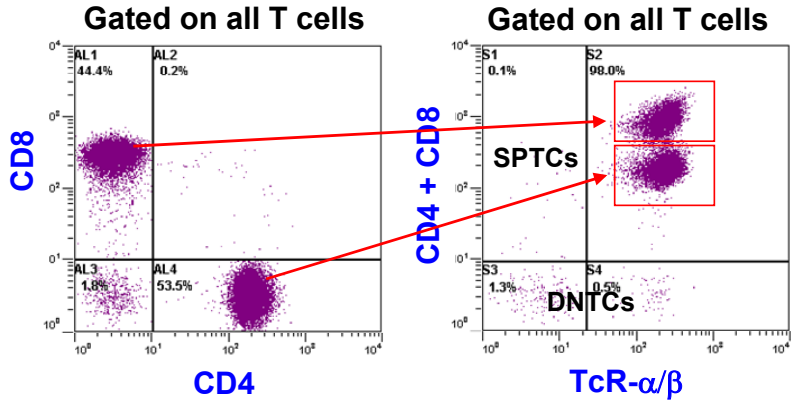

Figure 4a: Dichotomy of CD4 and CD8. Dotplot on the left shows the traditional way of depicting CD4+ and CD8+ T cells. Cells that are neither CD4 or CD8 positive can be found in the left lower quadrant. On the right, CD4 and CD8 are shown together to home in on single-positive T cells (SPTCs, upper quadrants) versus double-negative T cells (DNTCs, lower quadrants).

Julie Beach
Debbie Fearing

After the discovery of the CD4/CD8 dichotomy it became apparent that CD4 and CD8 are not the only two choices: a small population of DNTCs can be found in most humans. This is shown in a different way in the **right dotplot in figure 4a** (previous page). CD4 and CD8 are represented by the same FL channel on the **Y-axis**, while the **X-axis** corresponds to T cells expressing the alpha/beta T-cell receptor (α/β-TcR). Thus, DNTCs are found in the LL and LR quadrants, while single-positive T cells (SPTCs; since one cannot determine which cell population corresponds to CD4 or CD8) are in the upper quadrants. Based on the **X-axis**, T cells can be classified as being α/β-TcR-positive or α/β-TcR-negative (and thus gamma/delta TcR-positive, since T cells express either the α/β-TcR or the γ/δ-TcR).

DNTCs in humans are typically of the γ/δ-TcR variety. This is illustrated in **figure 4b**. The majority of cells are α/β-TcR+/SPTCs (either CD4+ or CD8+ T cells; **70.9%** in the UR quadrant). The large population below the horizontal quadrant marker is defined as a γ/δ-TcR+/DNTC population (LL quadrant; **24.9%** of T cells). This is also shown in a different format on the right (gated on all lymphocytes). The populations of cells in the left dotplot that remain to be defined are in the UL quadrant (γ/δ-TcR+/SPTCs; **7.6%** of T cells) and in the LR quadrant (α/β–TcR+/DNTCs; ~**1.1%** of T cells).

Thus, upon finding DNTCs, the first question to ask is whether they are gamma/delta T cells (more common) or alpha/beta T cells (less common).

DNTCs in the Context of the T-cell Receptor

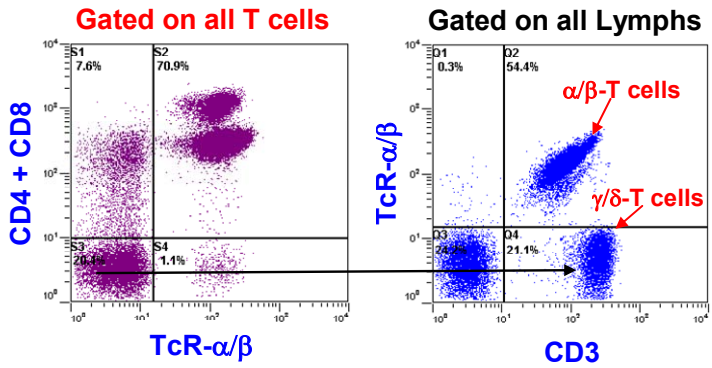

Figure 4b: Identification of the main SPTC and DNTC populations relative to carrying the alpha/beta versus the gamma/delta T-cell receptor.

The rare population of α/β-TcR+/DNTCs received notoriety when it was discovered that this T-cell subset is expanded in patients with autoimmune lymphoproliferative syndrome (ALPS). An example, obtained from an ALPS panel, is shown in **figure 4c** (next page). There are ALPS patients who show only small DNTC expansions (especially if immunosuppressive drugs are used). In addition, similarly expanded populations of α/β-TcR+/DNTCs can sometimes be found in healthy young children, as well as in patients with other immunological disorders. Distinguishing between these different scenarios can be difficult, but not impossible if additional immunophenotypic markers are included.

B220 (an isoform of CD45) is a particularly useful marker, as it appears to signify defective Fas-mediated apoptosis (the principle defect in ALPS). In **figure 4c**, several examples are shown of expanded α/β-TcR+/DNTC populations (**left dotplots**) in combination with B220 expression (**right dotplots**; gated on the cells in LR). The dotplots on top are from a patient with ALPS; the second set are derived from a patient with autoimmune manifestations, not on the basis of ALPS, while the third and fourth sets represent two patients with immunodeficiency disorders (a child with an undetermined T-cell defect and a child with DiGeorge syndrome, respectively). All dotplots were derived from the ALPS panel.

Immuno-101; further reading:

1. Cantor and Boyse. J. Exp. Med. 1975;141:1376.

2. Bernard and Bounsell. Hum. Immunol. 1984;11:1.

2. Bleesing et al. Clin. Immunol. 2002;104:21.

3. Ford et al. J. Exp. Med. 2002;196:261.

4. Thomson et al. Immunol. Res. 2006;35:163.

5. Bendelac et al. Annu. Rev. Immunol. 2007;25:297.

Ohio Buckeye in the Fall

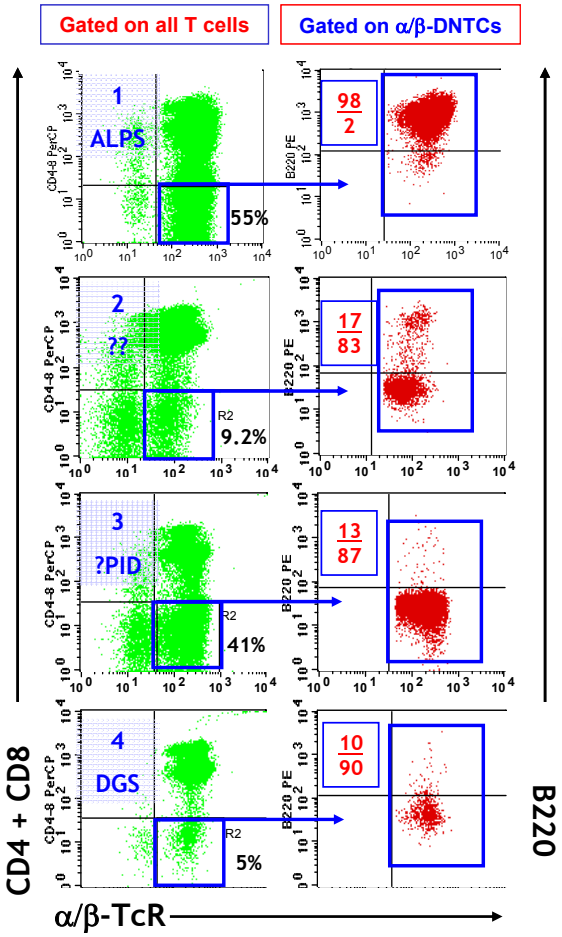

Figure 4c: α/β-TcR+/DNTCs in relation to B220 expression. Adding B220 and other markers (see sidebar) may help in distinguishing ALPS from other disorders with an expanded population of α/β-TcR+/DNTCs.

Taken in combination with the presence or absence of other immunophenotypic markers (e.g. HLA-DR, CD45RO), it appears that the origin (and perhaps disorder-specific relevance) of these DNTCs is different than those found in ALPS (e.g. extra-thymic differentiation versus end-stage T-cell differentiation and lack of apoptotic clearance).

There are other - equally rare - T-cell populations that can be found within the DNTC compartment (they may also have a counterpart within a SPTC compartment). For example, natural killer T cells (NKT cells) are defined by the expression of an α/β-TcR, defined in humans as Valpha24 and Vbeta11, in combination with NK-cell and other markers and sharing both "T-cell" and "NK-cell" functional characteristics. Other rare populations of DNTCs have been reported in mice. It remains unclear if these DNTCs exist in humans (see sidebar on page 7).

Lastly, it should be considered that DNTCs are in fact thymocytes inappropriately "caught" in peripheral blood at the double-negative state. Immunophenotypic markers that are specific for thymocytes, such as belonging to the CD1 family, in combination with markers that thymocytes typically do not express, can assist in distinguishing thymocytes from mature T cells.

Using a palette of immunophenotyping markers that focus both on absence and presence of cell surface and intracellular markers, including markers that may reflect pathogenesis (e.g. B220), as well as a more in-depth analysis of the immune system in general, one should be able to determine the identity of most (clinically relevant) DNTC populations and tie those populations to immunologic scenarios. As with all immunologic data, it is important to take age of the individual into consideration.

Helpful Immunophenotypic Markers to Distinguish Between DNTC Populations

- B220
- CD1
- CD27
- CD45RA
- CD45RO
- CD56
- CD57
- CD62L
- HLA-DR
- Perforin

Patient Vignette (part 2)

The adhesion marker assay is intended to screen for leukocyte adhesion deficiency type 1 (**LAD-1**). The laboratory finding in our patient showed a marked decrease in neutrophil CD11b/CD18 integrin expression, as well as a lack of integrin up-regulation, raising a strong concern for LAD-1.

LAD-1 is a rare primary immunodeficiency disorder caused by a defect in CD18. CD18 is the common β chain of β2 integrin family found predominantly on neutrophils. Due to the fact that β2 integrins are expressed on white blood cells in combinations of CD18 (β chain) and CD11 (α chain), abnormal CD18 expression is accompanied by a lack of CD11b expression, as demonstrated by our patient. Integrins are important for the attachment of various cells to other cells and tissues via specific receptors. In the case of the β2 integrins, the major function is to allow neutrophils to adhere to and migrate along endothelial cells and into the neighboring tissues.

Since neutrophils are important in the first line of defense against invading microorganism, the complete absence of β2 integrins causes a severe immunodeficiency by preventing neutrophil movement to sites of infection. The "classic" presentation of **LAD-1** is manifested by young infants and is characterized by a delayed separation of the umbilical cord, subsequent recurrent severe skin and mucosal infections with pyogenic microorganisms, and a lack of pus formation at sites of infection. β2 integrins are also relevant for T-cell and antigen-presenting cell function.

Patients display highly elevated blood neutrophil counts (that remain elevated even after infections have been brought under control). In the absence of allogeneic stem cell transplantation, life expectancy is very poor, with most patients not surviving beyond ten years of age.

A less common, moderate, phenotype of LAD-1 has been described in a handful of individuals. In these cases, CD18 is decreased, but not completely absent. Individuals with the moderate phenotype of **LAD-1** suffer from recurrent pyoderma gangrenosum-like skin ulcers, delayed wound healing, abnormal scarring, and only intermittently elevated blood neutrophil counts. Patients usually present in later childhood. Even though infections are not a prominent part of the initial disease course, affected individuals are prone to overwhelming infections. These case descriptions are identical to the clinical description of our patient.

In our patient, analysis of the gene encoding CD18 (***ITGB2***) showed a genotype consistent with **LAD-1** (and the observed CD11b/CD18 immunophenotype, as detected by the Adhesion Markers Assay). In summary, our patient best fits the category of "moderate" **LAD-1**. He presented with a rare manifestation of a rare disease, posing considerable diagnostic and therapeutic challenges. Moderate forms of **LAD-1 should be considered in patients with unexplained recurrent skin ulcers**. The flow cytometric assay demonstrated in this vignette provides a relatively straightforward diagnostic tool that can quickly screen for **LAD-1**.

The Golden Girls

LAD; further reading:

1. Ley et al. Nat. Rev. 2007;7:678.

2. Lekstrom-Himes and Gallin. N. Engl. J. Med. 2000;343:1703.

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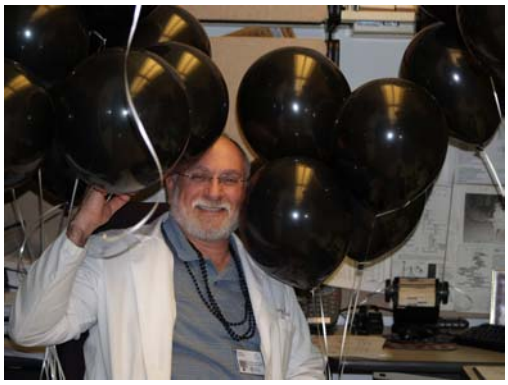
4. van de Kerkhof et al. Br. J. Dermatol. 1990;123:395.

5. Bedlow et al. Br. J. Dermatol. 1998;139:1064.

6. Davies et al. Clin. Exp. Immunol. 1991;84:223.



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).

RECAP

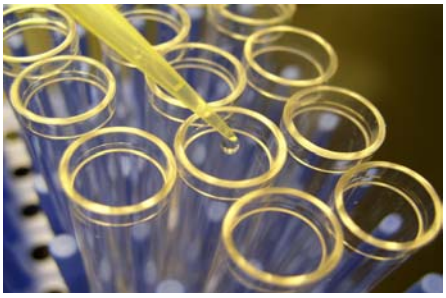
In this Newsletter, the following assays were discussed:

1] Adhesion Markers
A component in the battery of Neutrophil Function Tests, intended to screen for the presence of LAD-1. Other components of this battery include the Oxidative Burst Assay, which is a screening assay for chronic granulomatous disease (CGD), the Phagocytosis & Killing assay and the Chemotaxis assay.

2] BAFF Assay
Factor measured in serum (plasma) as part of B-cell reconstitution studies. See also **Spring 2007 Newsletter**.

3] CD107a Mobilization Assay
Assay in R&D phase to screen for the presence of familial HLH on the basis of mutations in MUNC13-4, and as a future component of NK-cell function studies (Granzyme B Transfer Assay). See also **Spring 2007 Newsletter**.

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



Recruitment

The Immunodeficiency and Histiocytosis Program in the Division of Hematology/Oncology has a variety of research protocols open for recruitment. These protocols share the main feature that they serve to better understand immunologic disease, as it pertains to increased susceptibility to infections, autoimmunity, lymphoproliferation and hemophagocytosis. Our research protocols focus on careful characterization of clinical disease combined with comprehensive immunologic and genetic testing of blood and/or tissues.

IN THE NEXT ISSUE

Patient Vignette

New Assays

Down the Pipeline

At the Interface

News from the Genetics Lab

Bulletin Board

CURRENT MENU OF AVAILABLE TESTS

Cincinnati Children's Hospital Medical Center

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

Test Requisition Form 091307

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

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

Test Name	Sample Requirement
❑ ALPS panel	3ml (1ml) EDTA ***CBC/Diff**
❑ Antigen Stimulation	10ml (5ml) Sodium Heparin
❑ B Cell Panel	3ml (1ml) EDTA ***CBC/Diff**
❑ Baff, plasma levels	3ml (1ml) EDTA
❑ CD40L (CD154)	5ml (3ml) Sodium Heparin
❑ CD45RA / CD45RO	3ml (1ml) EDTA
❑ CD64 (Leuko64)	1ml (0.5ml) EDTA
❑ CD132 / CD127	3ml (1ml) EDTA
❑ CTL function	10ml (5ml) Sodium Heparin
❑ Cytokines, Intracellular	3ml (2ml) Sodium Heparin
❑ Cytokines, Plasma	5ml (3ml) EDTA
❑ EBV immortalized cell line	3ml Sodium Heparin
❑ Check here if this is a research sample; signed consent required	
❑ ECP* (Eosinophil Cationic Protein)	3ml (1ml) serum*
Serum must be separated within 1 hr of collection. Call for details.	
❑ EDN (Eosinophil-derived neurotoxin)	3ml (1ml) EDTA
❑ Eotaxin-3	3ml (1ml) EDTA
❑ Foxp3	3ml (1ml) EDTA ***CBC/Diff**
❑ IL-13	3ml (1ml) EDTA

Test Name	Sample Requirement
❑ Lymphocyte Activation Markers	5ml (2ml) Sodium Heparin
❑ Lymphocyte subsets	3ml (1ml) EDTA ***CBC/Diff**
❑ Mitogen Stimulation	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	10ml (5ml) Sodium Heparin
❑ Perforin/Granzyme B	3ml (1ml) EDTA
❑ PNH Screen (CD59)	3ml (1ml) EDTA ***ON ICE***
❑ SAP protein (for R/O XLP)	3ml (1ml) Sodium Heparin
❑ Soluble IL-2R	3ml (1ml) EDTA
❑ TCR α/β / TCR γ/δ	3ml (1ml) EDTA
❑ TCR V beta clonogram	3ml (2ml) EDTA
❑ WASP	5ml (3ml) Sodium Heparin ***CBC/Diff**
❑ Other _____	

Diagnostic Immunology Laboratories

FOCIS Federation of Clinical Immunology Societies www.focisnet.org

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