



Great Lakes International Imaging and Flow Cytometry Association

GLIIFCA 15

September 29 - October 1, 2006

Marriott City Center

Pittsburgh, PA

www.gliifca.org

2006 Program Chairs:

Kathy Schell, President

Tim Bushnell

Paul Wallace

Site Organizer:

Alexander Nakeff

Local Hosts:

Vera & Albert Donnenberg

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GLIIFCA 15

Program

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GLIIFCA 15 GENERAL INFORMATION

(What You Always Wanted To Know About GLIIFCA 15 But Were Afraid To Ask!)

CONFERENCE REGISTRATION DESK: Grand Ballroom, Foyer B, 2nd Fl

Conference registration fee (\$80 early registration, \$95 on-site) includes Friday reception, Sat/Sun breakfast, Sat lunch, evening reception and banquet and coffee breaks.

- Friday, September 29 6pm – 10pm
- Saturday, September 30 8am – 8pm
- Sunday, October 1 9am – 12:30pm

POSTERS:

SET UP: Friday, September 29 after 5pm Grand Ballroom (GB) 4/5/6

- Numbers on posters correspond to poster abstract numbers in the program
- Poster board size= 3 ft wide and 4 ft high
- Please mount one poster on **each** side of a poster board using **VELCRO** only
- Viewing on Friday to Sunday 10:30am
- Presentation and Judging: Saturday 5:30-7p.

EXHIBITS:

- Scheduled exhibitors will have booths in the Exhibit/Poster area (GB 4/5/6)
- Booths will be open from 6p Friday to the end of the meeting
- All activities other than the plenary sessions, roundtable luncheon, Steering Committee meeting and banquet will be located in the Exhibit/Poster area (GB 4/5/6)
- **Please frequent the booths and show our appreciation for the generous financial support provided by exhibitors who substantially help "pay the freight" for this meeting**

BREAKFASTS:

- Free continental breakfast provided for all registrants in the Exhibit/Poster area (GB 4/5/6) on:
 - Saturday: 7:00 to 8:00am
 - Sunday: 8:00 to 9:00am
- Steering Committee breakfast meeting, Sunday morning (7:30 to 9am) in GB 1

COFFEE BREAKS:

- Snacks and drinks available in the Exhibit/Poster area (GB 4/5/6).

INDUSTRIAL SCIENCE SYMPOSIUM & FRIDAY RECEPTION

- Industrial Science Symposium presentations: 7-9p, Grand Ballroom 3
- Reception: 6-7p and 9-11p, GB 4/5/6
- Use drink tickets for wine/beer

SYMPOSIA LOCATION:

Grand Ballroom 3

SATURDAY LUNCHEON ROUNDTABLES (12:00– 2:00PM):

- Free “box” lunch/pop: ham/turkey and egg salad in Grand Ballroom 1/2. Sandwiches, chips and fruit at each roundtable of 10 – just pick up pop of choice and move to your roundtable of choice!

SATURDAY WINE AND CHEESE HAPPY HOUR:

- 5:30 – 7p in the Exhibit/Poster area (GB 4/5/6) with cheese and fresh fruit trays (use drink tickets).

BANQUET:

- Free to registrants and paid guests
- Commences at 8p, Grand Ballroom 1/2
- Numerous food stations (salad, entrée and dessert) provided to minimize waiting time
- Bar available for drinks (use drink tickets or pay cash)
- DJ with dance music until 12am; requests encouraged (get up and have fun!)

DRINKS:

- Bar located in the Exhibit/Poster area (GB 4/5/6) and banquet (GB 1/2)
- 4 free drink tickets/registrant for beer and wine only
- Bar prices: Beer: \$3.50; Wine - red & white (glass): \$4.25
- Mixed drinks – cash bar (your cost).
- All pop is free (ad libitum!)

FACILITIES/SERVICES:

- Parking: use valet service (your cost = \$23/day)
- Message boards: on easels next to the GLIIFCA Registration Desk
- Xerox copying, faxing, etc: Ask at Marriott registration desk

CMLE CREDITS:

- To receive CMLE credit, sign application form at Registration Desk.

NAME TAGS AND EVALUATION FORMS: Before leaving.....

- Fill out form and leave at Registration/Check Out Desk with your name tag.

ADDITIONAL ENQUIRIES:

- Contact Dr. Alexander Nakeff or Luellen Fletcher at the GLIIFCA registration desk.

GENERAL MEETING SCHEDULE:

All oral sessions will be held in Grand Ballroom (GB) 3. Poster viewing and Vendor Exhibits, breakfasts and breaks will be held in GB 4/5/6.GB Foyer B.

Friday, September 29

11a – 4p **Resource Managers Workshop**
City Center Room
Sponsored by: ISAC and GLIIFCA

6p – 11p **Poster Presentations**

7p – 9:00p **Industrial Science Symposium**
Grand Ballroom 3

6p – 11:00p **Opening Reception**
Grand Ballroom 4/5/6
Sponsored by: Beckman Coulter

Saturday, September 30

7:00a – 8:00a **Continental Breakfast**

8:00a – 11:00a **Symposium I**
Clinical Innovations

9:30 – 10:15a **Coffee Break**
Sponsored by: Spherotech

11:00a – 12:00 **The Carleton and Sigrid
Stewart Plenary
Lecture**

12:00-2:00p **Luncheon Roundtables**
Grand Ballroom 1/2

2:00p – 3:30p **Symposium II**
Imaging Innovations

3:30p – 4:00p **Coffee Break**
Sponsored by: Verity Software House

4:00p – 5:30p **Symposium III**
Cutting Edge

5:30p – 7p **Wine & Cheese Reception**
Sponsored by: iCyt Visionary Biosciences

5:30p – 7p **Poster Presentations and
Judging**

8p – Midnight **Banquet and Party**
Banquet Sponsored by: BD Biosciences
DJ Sponsored by: Beckman Coulter

Party Theme: Superheros of Cytometry
Costume Prize Sponsored by TreeStar

Sunday, October 1

8a – 9a **Continental Breakfast**

7:30a – 9a **Steering Comm. Meeting**
City Center B

9a – 11:45a **Symposium IV**
Application Innovations

10:30a – 11:00a **Coffee Break**
Sponsored by: Beckman Coulter

11:45a–12:00p **Poster Award**
Sponsored by: Beckman Coulter (2) and
Jackson ImmunoResearch Labs (2)

Travel Stipend Awards
Sponsored by: Amnis (3,)Beckman Coulter
(2), Cell Signaling Technology (4) and
Cytonome

12:00p – 12:15p **News for Next Year and
Meeting Wrap Up**

BEFORE LEAVING: Return evaluation form and name tag holder to Registration/Check out Desk. Thank you!!!!

Speaker Schedule – At a Glance

Resource (Core) Managers Workshop

Friday, September 29 11:00-4:00pm
Conveners: R. Duggan & J. Moore

Industrial Science Symposium

Friday, September 29 7:00 – 9:00pm
Convener: A. Nakeff & B. Pesch

- 7:00-7:30 *Ruud Hulspas, Cytonome*
The Next Phase in Clinical-grade, High-speed Cell Sorting: Parallel Microfluidic Optical Sorters on a Chip
- 7:30-8:00 *David Cheo, BD Biosciences*
High Content Imaging: Taking Cell Analysis to a Higher Resolution
- 8:00-8:30 *David Basiji, Amnis Corp.*
Thinking Outside the Dot: Quantitative Cell Image Analysis in Flow with the ImageStream System
- 8:30-9:00 *Maria Daly, Beckman Coulter*
Automation of Bead-based Flow Cytometric Analysis to Measure 10-plex Cytokine Arrays in a 96-well Plate Format

Symposium I – Clinical Innovations

Saturday, Sept 30 8:00 am – 11:00 am
Conveners: K. Domenico & P. Wallace

- 8:00 – 8:45 *Thomas Shanahan, Upstate NY Transplant Services*
The Flow Cytometry Approach to Transplant Compatibility
- 8:45 – 9:30 *Raymond Felgar, U of Rochester*
Application of Flow Cytometry to the World Health Organization (WHO) Classification of Hematologic Malignancy: A Hematopathology Perspective

9:30 – 10:15 Coffee Break

- 10:15 – 11:00 *George Deeb, Roswell Park Cancer Institute*
Multiparameter Flow Cytometry in Hematopathology: Case Studies with Clinicopathologic Correlation

The Carleton and Sigrid Stewart Plenary Lecture

11:00 – 12:00

Alan Waggoner, Carnegie Mellon Univ
Fluorescent Probes and Imaging Live Cells

Luncheon Roundtables

12:00 – 2:00

Convener: M. Paniagua
Sign-up sheets at Registration Desk

Symposium II – Imaging Innovations

2:00 pm – 3:30 pm

Convener: V. Donnenberg

- 2:00 – 2:45 *Simon Watkins, U of Pittsburgh*
Live Cell Imaging Shows Novel Functional Interconnections between Cells of the Immune System
- 2:45 – 3:30 *Robert Murphy, Carnegie Mellon Univ*
Automated Interpretation and Modeling of Subcellular Patterns for Systems Biology
- 3:30-4:00 Coffee Break**

Symposium III – Cutting Edge

Saturday, Sept 30 4:00 pm – 5:30 pm
Convener: J. Moore

- 4:00 – 4:45 *Avinash Bhandoola, U of Pennsylvania*
From Stem Cell to T Cell: One Route or Many?
- 4:45 – 5:30 *J. Philip McCoy, NHLBI, NIH*
Circulating Endothelial Cells: Rare and Well-Done

5:30 – 7:00 Poster Presentations and Judging

Symposium IV – Application Innovations

Sunday, Oct 1 9:00 am – 11:45 am

Convener: T. Bushnell

- 9:00 – 9:45 *William Telford, NCI*
Novel Excitation Sources for Flow and Image Cytometry
- 9:45 – 10:30 *Timothy Ragan, MIT*
3D Tissue Cytometric Approaches to Tissues and Whole Organs
- 10:30 – 11:00 Coffee Break**
- 11:00 – 11:45 **Young Investigator Talk**
Lori Broderick, Univ at Buffalo
IL-12/TGF β 1 Regulation of TCR Signal Transduction in Human Lung Tumor-Associated Memory T Cells

Industrial Science Symposium
Friday, Sept 29th
7pm – 9:00pm
Grand Ballroom 3

Conveners: Bruce Pesch, Nat'l Animal Disease Ctr & Alexander Nakeff, Henry Ford Health System

7:00 – 7:30 The Next Phase in Clinical Grade, High-Speed Cell Sorting: Parallel Microfluidic Optical Sorters on a Chip

Rudd Hulspas, Cytonome, Inc.

7:30 – 8:00 High Content Imaging: Taking Cell Analysis to a Higher Resolution

David Cheo, BD Biosciences

8:00 – 8:30 Thinking Outside the Dot: Quantitative Cell Image Analysis in Flow with the ImageStream System

David Basiji, Amnis Corporation

8:30 – 9:00 Automation of Bead-Based Flow Cytometric Analysis to Measure 10-plex Cytokine Arrays in a 96-well Plate Format

Maria Daly, Beckman-Coulter/Immune Tolerance Network

Abstracts:

The Next Phase in Clinical Grade, High-Speed Cell Sorting: Parallel Microfluidic Optical Sorters on a Chip

Rudd Hulspas, Cytonome, Inc

Obtaining sufficient purified cells for therapeutic applications is one of the challenges in clinical flow sorting. In addition, conventional high-speed droplet sorters inflict a high risk of sample contamination and exposure to hazardous material. Microfluidic switch sorting does not require droplet formation and thus can take place in entirely enclosed systems. Microfluidic switch sorting, however, has been slow compared to high-speed and even conventional droplet sorters. Two important recent developments have dramatically increased the overall cell sort rate in microfluidic switch sorting: (1) a 10-fold increase in sort rate and (2) parallel implementation of multiple switches on a single chip. This technology will overcome many limitations in cell processing speed, selection capability and cGMP compliance and will enable a host of new cell therapies that are not possible at this time.

High Content Imaging: Taking Cell Analysis to a Higher Resolution

David Cheo, BD Biosciences

High content imaging is rapidly becoming a mainstay in pharmaceutical and life-science research laboratories. The power of imaging resides in its ability to measure not only fluorescence intensity changes within cells, but also molecular redistribution and morphological features of cells and tissues in a 3D environment. In recent years, the availability of a new generation of automated high content imaging platforms has created a unique opportunity to develop novel cell-based assays. To be used effectively in cell analysis, an automated imaging system must provide a wide range of capabilities including live-cell kinetic imaging, endpoint imaging and high-resolution confocal imaging. These features of the BD Pathway Bioimager, coupled with versatile user

configurable image data acquisition, processing and analysis software, allow the researcher to make multi-parameter measurements of cellular events in multi-dimensional space and time. This presentation will highlight a number of specific applications developed for the BD Pathway system that demonstrate its effectiveness as a powerful analytical cell biology research tool.

Thinking Outside the Dot: Quantitative Cell Image Analysis in Flow with the ImageStream System

David Basiji, *Amnis Corporation*

This presentation will provide an introduction to the technology and applications of the ImageStream(r) system for imaging in flow. A portion of the talk will describe the core technologies used by the ImageStream to generate image data. The remainder of the talk will focus on two example applications, nuclear translocation and the analysis of changes in cell morphology. In the analysis of NF-kB translocation, we will show how the molecular distribution may be quantitated, and we will also discuss some of the novel features in the IDEAS(r) image analysis package that facilitate the quantitative comparison of image channels. Quantitation of cell morphology change will be presented using pseudopod formation as a representative example. In this application we will show how a cell population may be subdivided using structural criteria alone and how particular cell morphologies may be correlated with other factors, such as molecular distribution.

Automation of Bead-Based Flow Cytometric Analysis to Measure 10-plex Cytokine Arrays in a 96-well Plate Format

Maria Daly, *Beckman-Coulter/Immune Tolerance Network*

Studying large groups of patients for subtle changes in T Cell proliferative activity and cytokine expression has many potential problems, issues such as reproducibility and quality control are paramount. These issues when combined with the usual assay requirements, such as specificity and sensitivity, automation of sample preparation is an obvious route to pursue. However, cells in suspension and bead based assays are relatively new to liquid handling and so this technology itself presents challenges. In this session aspects of preparing a technique for automation will be discussed, and data gained will be presented.

Symposium I
Saturday, Sept 30th
8:00 am – 11:00 am
Grand Ballroom 3
Clinical Innovations

Conveners: Kathy Domenico, The Medical University of Ohio & Paul Wallace,
Roswell Park Cancer Institute

8:00 – 8:45 The Flow Cytometry Approach to Transplant Compatibility
Thomas Shanahan, Upstate NY Transplant Services

**8:45 – 9:30 Application of Flow Cytometry to the World Health Organization
(WHO) Classification of Hematologic Malignancy: A
Hematopathology Perspective**
Raymond Felgar, University of Rochester

9:30 – 10:15 Coffee Break – Posters and Exhibits

**10:15 – 11:00 Multiparameter Flow Cytometry in Hematopathology: Case Studies
with Clinicopathologic Correlation**
George Deeb, Roswell Park Cancer Institute

Abstracts:

The Flow Cytometry Approach to Transplant Compatibility
Tom Shanahan, Upstate NY Transplant Services

Transplantation is the preferred treatment for many cases of end-stage organ disease. But for some patients, allo-reactive antibodies provide a major barrier to successful outcomes. These antibodies, primarily HLA specific, mediate irreversible forms of transplant rejection. For this reason, their detection in the pre-transplant period is crucial to patient well-being.

An additional challenge in this regard is the fact that many transplant candidates exhibit benign antibody reactions that mimic clinically significant ones. Although the antibodies in these instances are incapable of graft destruction, they may needlessly preclude some patients from this life saving treatment.

Flow cytometry offers a valuable approach to the resolution of this dilemma. The sensitivity of the assay allows the detection of the clinically relevant antibodies; the specificity of the procedure permits their identification and classification. Therefore, the flow cytometric assessment of transplant compatibility ensures the most favorable outcomes while making certain that no transplant candidate is unfairly excluded from consideration for this valuable but limited resource.

Correspondence: tshanahan@unyts.org

**Application of Flow Cytometry to the World Health Organization (WHO)
Classification of Hematologic Malignancy: A Hematopathology Perspective**
Raymond Felgar, University of Rochester

Advances in the understanding of the biology of lymphomas and leukemias have led to several major revisions of classification schemes. The most recent of these is the current WHO classification, which attempts to correlate most diseases to the most likely normal cell counterpart and/or stage of differentiation. Immunophenotyping by flow cytometry, immunohistochemistry, in situ hybridization, and other methods has played a particularly integral part in making an accurate diagnosis. Flow cytometry evaluation especially has been useful in rapidly diagnosing leukemias and is often integral to the diagnosis of lymphomas. Advantages of flow cytometry over some of the other methods are (1) rapid analysis of large numbers of cells, (2) increased sensitivity of antigen detection, and (3) ability to look at multiple antigens simultaneously on a single cell population. In particular, the use of 4, 5, and 6 color flow has increased our ability to find subtle abnormalities in expression profiles. Another advantage of multi-color analysis is the ability to look at multiple markers on samples with relatively low cell counts, such as cerebrospinal fluid samples and fine needle aspirate samples. We will review some of the more common methods for gating and analysis, along with discussion of appropriate panel design for detecting the more common malignancies (precursor T and B cell leukemia, acute myelogenous leukemia, T-cell malignancy, cytoplasmic antigen assessment, light scatter vs. CD45 based gating, limited use of other markers such as CD19 or CD20 for gating, Boolean gating strategies in select cases). Where appropriate, specific examples will be used to illustrate key points. This lecture is designed to serve as a staging ground for the lecture to follow (given by Dr. Deeb who will be illustrating specific case studies in leukemia and lymphoma).

Correspondence: Raymond_Felgar@urmc.rochester.edu

Multiparameter Flow Cytometry in Hematopathology: Case Studies with Clinicopathologic Correlation

George Deeb, Roswell Park Cancer Institute

Immunophenotyping by Multiparameter Flow Cytometry has a significant role in diagnosis and classification of hematopoietic malignancies. To highlight the utility of this modality in daily hematopathology practice, several clinical cases will be discussed with emphasis on the design of antibody combinations, the gating strategy, and the immunophenotypic characteristics of the different cellular populations in the analyzed samples. The discussion will include clinical, histomorphologic, and molecular correlation.

Correspondence: George.Deeb@Roswellpark.org

**The Carleton and Sigrid Stewart Plenary Lecture
Saturday, September 30
11:00 am – 12:00 pm
Grand Ballroom 3**

Alan Waggoner, Ph.D.

**Director, Molecular Biosensor and Imaging Center
Professor of Biological Sciences and Biomedical Engineering
Carnegie Mellon University**

Fluorescent Probes and Imaging Live Cells

The goal is to provide a good overview of the use of fluorescence detection technologies in cytometry. The presentation will emphasize the fundamentals of fluorescent probes in flow cytometry and fluorescence imaging including the latest fluorescent probe technologies. It will touch on the basics of photo-physics, the excited state of fluorescent dyes, fluorescent labels, fluorescent physiological indicators, energy transfer, fluorescence polarization, two-photon imaging, fluorescence detection components and instrumentation and signal-to-noise issues.

Correspondence: waggoner@andrew.cmu.edu

Luncheon Roundtables
Grand Ballroom 1/2
Saturday, Sept 30 12:00-2:00pm
Convener: Mary Paniagua, University of Chicago

Free box lunch (assorted varieties) available at each table – drinks available at side tables.
Please sign up for these sessions at the Registration Desk.

The Roundtable sessions serve as informal discussion groups conducted over lunch. The primary aim of these discussions is to provide a forum for participants to address their interests in a variety of cytometry and image topics.

1 - Imaging Flow Cytometry: Visualizing flow-based assays of immunity

Chair: Albert Donnenberg, University of Pittsburgh

2 - Laser excitation sources for flow and image cytometry: Updates on the latest laser technology and the implications of their availability for instrumentation upgrades and assay implementation.

Chair: Bill Telford, National Cancer Institute

3 - Leukemia/Lymphoma discussion group: A two-part discussion including update on latest issues relating to flow cytometric immunophenotyping of leukemias and lymphomas as well as clinicopathologic correlation of phenotype with histology

Co-chairs: Paul Wallace and George Deeb, Roswell Park Cancer Institute

4 - Polychromatic Flow Cytometry—Beyond Immunophenotyping: discussion of the challenges of using a range of indicator dyes (live/dead discrimination, Qdots, autofluorescence) in addition to monoclonal antibodies for multicolor flow cytometry.

Chair: Matthew Hanson, University of Wisconsin

5 - Transplant compatibility assessment: Overview of flow cytometry applications for the evaluation of clinically significant allo-reactive antibodies in solid organ transplantation.

Chair: Tom Shanahan, Upstate New York Transplant Services

6 - Cancer Stem Cell Evaluation: Application of polychromatic flow cytometry to the cancer stem cell paradigm

Chair: Vera Donnenberg, University of Pittsburgh

7 - Working with Digital Flow Cytometry Data: discussion of digital error, its effect on compensation, and the requirement for Biexponential/Hyperlog displays

Chair: Mark Munson, Verity Software House

8 - Do-it-Yourself Service: Focus will be alternatives to traditional service contracts for cytometry instrumentation, i.e. self-service, third party, parts only—as well as advantages/disadvantages to this approach

Co-chairs: Ryan Duggan, University of Chicago and Alex Rodriguez, Case Western Reserve University

9 - Clinical Flow Cytometry Basics: Operations and management issues relevant to the clinical flow cytometry laboratory including but not limited to inspections, proficiency testing, instrument selection and evaluation, quality control and common applications.

Co-chairs: Karen Domenico and Tom Sawyer, Medical University of Ohio

10 - Instrument Performance Validation: We all generate lots of cytometry data from day to day but are we generating data that accurately reflects our samples? Understanding your instruments limits of detection as well as available tools for verifying an instrument's performance will be central to this discussion.

Chair: Joanne Lannigan, University of Virginia

11 - 25 Years Later--The State of HIV Assays in the Clinical Lab: A discussion of new technologies in CD4 counting

Chair: Frank Mandy, Natl. HIV and Retrovirology Laboratories, Public Health Agency of Canada

Symposium II
Saturday, Sept 30
2:00 pm – 3:30 pm
Grand Ballroom 3
Imaging Innovations

Convener: Vera Donnenberg, U Pittsburgh

2:00 – 2:45 Live Cell Imaging Shows Novel Functional Interconnections between Cells of the Immune System

Simon Watkins, University of Pittsburgh

2:45 – 3:30 Automated Interpretation and Modeling of Subcellular Patterns for Systems Biology

Robert F. Murphy, Carnegie Mellon University

3:30 – 4:00 Coffee Break – Poster & Exhibits

Abstracts:

Live Cell Imaging Shows Novel Functional Interconnections between Cells of the Immune System

Simon Watkins, and Russ Salter, University of Pittsburgh

Dendritic cells are ubiquitous throughout the body, their primary function is as “danger” detectors, the cells take up antigens from exogenous agents, process and present the antigen on the surface of the cell. Once exposed to antigen the cells mature, and migrate through the lymphatic drainage system to the lymph node where they present the antigen to effector cells of the immune system (such as cytotoxic T lymphocytes). In this study we show that rather than acting in isolation, the DCs are actually physically interconnected through a network of extremely small tubules which allows clonal activation of populations of cells rather than each cell acting independently. Essentially electrical connectivity between cells allows a single cell to activate several neighbors (over a distance of several hundred microns). These activated cells then migrate toward the signaling cell and take up and process antigen also. Once activated the cells lose their dendritic appearance prior to migrating through the lymphatics. Once at the lymph node they reassume their dendritic morphology. This study depended critically on an array of live cell imaging methods; ratiometric measures of calcium concentration, high resolution DIC, Live cell Confocal, Confocal, 2P methods as an integrated imaging solution we have been able to define the functionality of a novel and hitherto undescribed method of intercellular communication which will be presented in this talk.

Correspondence: swatkins@pitt.edu

Automated Interpretation and Modeling of Subcellular Patterns for Systems Biology

Robert F. Murphy, Carnegie Mellon University

Proteomics will play a critical role in systems biology efforts to build accurate models of entire eukaryotic organisms. Simply identifying which of tens of thousands of proteins

are expressed in each of over a hundred animal cell types is a daunting task. To meaningfully understand the behavior of even a single cell type, we need information on not only which proteins are expressed but within what subcellular structures they are found. The information must also be placed into a systematic framework, but assignments of standardized terms by curators do not always result in the same terms being assigned to proteins that have the same pattern. Further, these terms do not have the richness to capture high-resolution locations such as “the rims of recycling tubules emanating from endosomes.” Fortunately, advances in microscopy and machine learning provide an alternative. Our group has demonstrated that automated methods can discriminate subcellular patterns that cannot be distinguished by visual examination. These methods are based on sets of Subcellular Location Features that capture the essence of patterns without being overly sensitive to cell size, shape and orientation. We have also demonstrated that cluster analysis can be used to group fluorescently-tagged proteins by their high-resolution location patterns. The results show that not only are highly similar patterns grouped together automatically without human assistance, but that proteins that humans lump together (such as different subpatterns of cytoplasmic proteins) are properly separated. In addition, we have obtained encouraging results on building generative models of subcellular patterns for use in cell simulations. These models can be captured in XML fragments and allow images (distributions) to be generated that reflect the statistical variation in pattern within a particular location family. Our results suggest the value of combining automated analysis with large scale protein tagging to define the patterns that proteins can occupy in different cell types, an approach we term location proteomics.

Correspondence: murphy@andrew.cmu.edu

Symposium III
Saturday, Sept 30
4:00 pm – 5:30 pm
Grand Ballroom 3
Cutting Edge

Convener: Jonni Moore, University of Pennsylvania

4:00 – 4:45 From Stem Cell to T Cell: One Route or Many?

Avinash Bhandoola, U of Pennsylvania

4:45 – 5:30 Circulating Endothelial Cells: Rare and Well-done

Phil McCoy, NHLBI, NIH, Bethesda

Abstracts:

From Stem Cell to T Cell: One Route or Many?

Avinash Bhandoola, U of Pennsylvania

The identity of bone marrow progenitors that physiologically seed the adult mouse thymus is unknown. We have previously examined progenitors in the blood of adult mice, and have shown that a very rare population of cells with a Lineage- Sca-1(hi) c-Kit(hi) (LSK) phenotype circulate. This LSK population includes hematopoietic stem cells (HSCs) and downstream multipotent progenitors (MPPs), which are efficient T cell progenitors. We have now examined whether these progenitor subsets are able to settle in the thymus from the blood. We find that whereas HSCs and MPPs generate T cells with comparable efficiency when injected intrathymically, only MPPs rapidly generate T cells upon intravenous transfer into unirradiated mice. This result differs from previous studies using irradiated recipient mice, and indicates that MPPs selectively settle in the thymus in the physiological situation. We find that the chemokine receptor CCR9 is expressed by a subset of MPPs but not by HSCs, and is functionally important for thymic settling by MPPs in unirradiated recipient mice. We conclude that whereas multiple bone marrow progenitors have T lineage potential when injected intrathymically, some progenitors do not efficiently settle within the thymus under physiological conditions. MPPs are likely to constitute the principal progenitors of T cells in adult mice, but there may be multiple routes connecting HSCs in the bone marrow and T cells in the thymus.

Correspondence: bhandooa@mail.med.upenn.edu

Circulating Endothelial Cells: Rare and Well-done

J Philip McCoy, Jr., NHLBI, NIH, Bethesda

The endothelium, the lining of blood vessels, is one of the largest and least understood structures in the human body. It regulates trafficking of cells and material from the blood into tissues as well as contributing to the structural integrity of the vessels. In various diseases perturbations to the endothelium can occur, however these are difficult to assess as the endothelium is not easily studied in vivo or biopsied. Endothelial cells have been detected in the peripheral circulation and may arise from either endothelial precursors or shed endothelium. These circulating endothelial cells (CECs) are currently being studied

in diseases such as cancer and coronary heart disease where it is thought they provide a biomarker of angiogenesis. In other diseases such as sickle cell anemia they may be an indicator of vascular damage. The measurement of CECs is quite challenging. In healthy individuals, CECs are present in extremely low concentrations – generally around 10-20 cells per ml of blood. Performing accurate enumeration of these cells requires implementation of procedures to optimize detection of rare events. Among these procedures are a thorough cleaning of the cytometer prior to analysis, the use of a ‘dump’ channel to identify and gate out non-CECs, eliminating staining of dead cells with viability stains, and collecting large data files. The lack of highly specific markers for endothelium also contributes to the difficulty in CEC enumeration. Examples of flow assays for CEC enumeration will be shown as well as assays for endothelial progenitor cells

Correspondence: mccoyj@nhlbi.nih.gov

Symposium IV
Sunday, Oct 1
9:00 am – 12 noon
Grand Ballroom 3
Application Innovations
Conveners: Timothy Bushnell, University of Rochester

9:00 - 9:45 Novel Excitation Sources for Flow and Image Cytometry
William Telford, National Cancer Institute

9:45 – 10:30 3D Tissue Cytometric Approaches to Tissues and Whole Organs
Timothy Ragan, Massachusetts Institute of Technology

10:30 – 11:00 Coffee Break

11:00 – 11:45 Young Investigator Talk
IL-12/TGF- β 1 Regulation of TCR Signal Transduction in Human Lung Tumor-Associated Memory T Cells
Lori Broderick, University at Buffalo

Abstracts:

Novel Excitation Sources for Flow and Image Cytometry
William Telford, National Cancer Institute

Laser excitation sources covering virtually the entire visible spectrum are rapidly becoming available and can be integrated into cytometric instrumentation. The availability of a wide variety of laser wavelengths permits a very different paradigmatic approach to how we do flow cytometry. By eliminating the constraints of traditional laser choices, we can stop designing assays and choosing methods based on the limitations of our instruments, and instead redesign our instruments to meet the needs of our assays. This seminar will provide an update on latest laser technology available for flow cytometry, and how it can be implemented into commercial instrumentation. A number of novel fluorescent assay systems previously inaccessible with conventional laser sources will also be described.

Correspondence: telfordw@mail.nih.gov

3D Tissue Cytometric Approaches to Tissues and Whole Organs
Timothy Ragan, Massachusetts Institute of Technology

We have developed a high throughput tissue cytometer based around a high-speed 3D two-photon microscope. Unlike traditional slide cytology, our approach is particularly well suited for thick tissues as it allows quantitative 3D observations of cellular biochemistry and morphology across a statistically significant (10^6) population of cells in their native tissue environment.

In addition, we have integrated an automated microtome into the system to circumvent the depth limitation inherent in optical microscopy. By alternating optical and mechanical sectioning it is possible to ex vivo image entire organs in small mammals and perform cytometric measurements on every cell in an organ. This opens up an entire class of applications previously impossible or too time intensive to contemplate. We

present data on cytometric measurements of metastasis cells in macroscopic portions of liver as well as whole heart physiology at the cytometric level.

Correspondence: tragan@mit.edu

IL-12/TGF- β 1 Regulation of TCR Signal Transduction in Human Lung Tumor-Associated Memory T Cells

Lori Broderick, *University at Buffalo*

Memory T cells in the microenvironment of human non-small cell lung tumors fail to respond to activation signals through the T cell receptor (TCR). This arrest in T cell signaling is due to a blockade in the proximal TCR signaling cascade and is mediated by TGF- β 1 that is expressed on the membrane of cells in the tumor microenvironment. We will demonstrate that this T cell anergy can be reversed in vitro by a brief pulse of the tumor-associated T cells with IL-12. Using a human lung tumor xenografts model it has been established that the TCR arrest can be reversed in situ by a local and sustained release of IL-12 into the microenvironment of human tumor xenografts. The cytokine reversal of the T cell anergy results in the activation, proliferation and T cell mediated eradication of tumor cells from the xenografts.

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POSTER ABSTRACTS

Abstract number corresponds to poster board number.
All posters displayed in Exhibit area – Grand Ballroom 4/5/6

#1. Prostaglandin E2 downregulates the expression of CD11c in dendritic cells.

Jui-Hung Yen¹, Doina Ganea²: ¹Rutgers University, Newark, NJ, ²Temple University School of Medicine, Philadelphia, PA

Dendritic Cells (DCs) play an important role in the initiation of the immune response. Immature DCs take up antigen, mature and migrate to the regional lymph nodes where they activate antigen-specific naïve T cells. Prostaglandins such as PGE₂ are essential for the migration of DCs to the lymph nodes. CD11c, a hallmark of DCs, is an integrin constitutively expressed on DCs. Although CD11c is used as a DCs marker, its physiological role is not clear. Here we report that PGE₂ down-regulates CD11c expression at mRNA and protein levels in DCs, and that this down-regulation can be reversed by DC-T cell interactions. The effect of PGE₂ is mediated through the EP-2/EP-4 receptors expressed on DCs. Downstream of the EP-2/EP-4 receptors, cAMP and PKA but not EPAC are involved in the down-regulation of CD11c. When PGE₂-treated DCs are cultured with TCR-transgenic T cells in the presence of the specific antigen, CD11c on DC expression is restored. Although previous studies indicated that PGE₂ is essential for the migration of mature DCs to the regional lymph nodes, the molecular mechanisms have not been elucidated. We propose that the effects of PGE₂ on CD11c expression and DC migration are functionally linked. According to our model, the PGE₂-induced down-regulation of the CD11c integrin in the peripheral tissues is responsible, at least partially, for promoting DCs migration to the regional lymph nodes. In contrast, following arrival into the lymph nodes, initial contacts with T cells restore CD11c expression, allowing the stronger and prolonged DC/T cell contacts that lead to the activation of antigen-specific T cells.

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#2. It's Not Just for Mice: A Practical Guide to Identifying and Sorting "SP" Cells From Various Cell Sources.

William Murphy, Andrew D. Bantly, Charles H. Pletcher, Jr., Jonni S. Moore. Abramson Cancer Center Flow Cytometry and Cell Sorting Resource Laboratory, Path BioResource, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA

GOAL: The isolation, identification, and characterization of progenitor cells from virtually all tissue types and across species has become the focus of many investigations. Often these populations are not well defined by surface protein expression so investigators must rely on functional assays, such as the identification of the Hoechst "side population" (SP cells). We sought to define critical parameters for the performance of the SP assay using various cell sources and across instrument platforms.

BACKGROUND: The therapeutic potential of adult stem cells is being widely investigated and has led to increased demand for methods to identify and/or isolate potential candidate populations. Goodell and co-workers (1996) described a method to identify putative progenitors in murine bone marrow based on extrusion of Hoechst 33342 via an efflux pump. Identification of this exceedingly small population, using this approach, has proven to be useful in the absence of definitive surface markers, however is a technically challenging assay. In order to provide scientifically sound data, it is critical

to understand the conditions that affect this assay and how to accurately and reproducibly detect these very rare cells.

METHODS: By analyzing the data from a variety of cell sources, we determined critical aspects of SP cell identification could be focused in three areas: 1) cell preparation/ labeling; 2) instrument set up/qc; 3) data analysis/controls. Each area is addressed using examples demonstrating importance of dye titration, timing, cell concentration, gating controls etc. Details of assay performance on several platforms (BD LSRII, BD FACSVantage SE DiVa) and with several lasers (355mw Coherent water cooled UV, 355mw Lightwave solid state UV, and a 405mw solid state violet) are presented. Using these approaches, SP cells can be reproducibly identified and sorted from multiple sources and provide the basis for identification of multiple types of adult stem cells.

#3. Time-gated luminescence flow cytometry. *Dayong Jin, Russell Connally and James Piper. Centre for Lasers & Applications, Division of Information & Communication Sciences, Macquarie University, NSW 2109 Australia*

Flow cytometry is a process in which light scattering or fluorescence measurements are taken as cells passage through a defined excitation/interrogation zone, preferably in single file in a fluid stream. Flow cytometers can identify and enumerate target cells on a continuous basis at rates of 1000 to 40,000 cells per second. For the last two decades, flow cytometry has been suggested as a more effective method than fluorescence microscopy for the purpose of quantifying microbial cells, e.g., monitoring of bacteria and other microorganisms in water, beverage, milk and food, with the prospect of rapid real-time analysis.

The application of flow cytometry for the accurate detection of target microorganisms (<10 μ m diameter) within an intrinsically fluorescent matrix of non-target particles, e.g., minerals, plant debris and algae, often requires multi-color fluorescence staining followed by multi-spectrum detection. The complexity and cost of such techniques is significant and detection accuracy is not guaranteed, particularly when the target organism is in very low concentration, e.g. <100 cells in 10 L water.

I will present experimental and theoretical studies of time-gated discrimination of long-lived luminescence (lifetime: 1~2000 μ s) labeled target-organisms against non-target autofluorescence background (lifetime: <100 ns) in flow cytometry. When periodically illuminating the excitation zone, autofluorescence rapidly fades within 1 μ s once the excitation pulse has extinguished whilst the luminescence from target organisms/cells persists as cells progress downstream for a given distance, typically hundreds of microns. This technique offers the opportunity for time-delayed detection of target organisms in a region spatially disconnected from the excitation zone with (theoretically) zero background. Remarkably, ultraviolet (UV) light emitting diodes (LEDs), once filtered, were found compatible as excitation sources for our TGL flow cytometer.

The TGL flow cytometer was successfully constructed and optimized to operate at 6 kHz repetition rate of TGL cycles consisting of 100 μ s LED excitation and ~60 μ s gated high-gain detection using a channel photomultiplier. The spatial counting efficiency was evaluated by enumerating 5.5 μ m diameter europium microspheres resulting in a counting accuracy approaching 100%. The instrument was then employed for the real-time rapid counting of a rare-event microbial pathogen cell, *Giardia* cyst (6 μ m ~ 9 μ m diameter) (immunofluorescently labeled with europium chelates previously), with concentration of <100 events in 200 μ L environmental contaminated water concentrate containing a large number of non-target intrinsic-autofluorescent particles. The effective

suppression of autofluorescent background rendered non-target particles invisible to the time-delayed detection phase providing an ultra-low (0.2 volts) background when contrasted against signal pulses (maximum of 4.6 volts). A simplified and accurate means of counting rare-event microorganisms was possible following suppression of prompt fluorescence. The prototype instrument achieved accurate detection of rare pathogenic cells against a strongly fluorescent background. Such an instrument has the potential to operate as a portable TGL flow cytometer that eliminates the complex multi-color immunofluorescence labeling required for detection on conventional flow cytometers.

#4. Isolating gene-corrected stem cells without drug selection. *Larry W. Arnold*+++, Seigo Hatada+, Tomoko Hatada+, John E Cowhig+, Dominic Ciavatta+, and Oliver Smithies++++. Departments of *Microbiology and Immunology and +Pathology and Laboratory Medicine, and ++Lineberger Comprehensive Cancer Center, University of North Carolina..*

Progress in isolating stem cells from tissues, or generating them from adult cells by nuclear transfer, encourages attempts to use stem cells from affected individuals for gene correction and autologous therapy. Current viral vectors are efficient at introducing transgenic sequences but result in random integrations. Gene targeting, in contrast, can directly correct an affected gene, or incorporate corrective sequences into a site free from undesirable side effects, but efficiency is low. Most current targeting procedures, consequently, use positive-negative selection with drugs, often requiring ≥ 10 days. This drug selection causes problems with stem cells that differentiate in this time or require feeder cells, because the feeders must be drug resistant and so are not eliminated by the selection. To overcome these problems, we have developed a procedure for isolating gene-corrected stem cells free from feeder cells after 3-5 days culture without drugs. The method is still positive-negative, but the positive and negative drug-resistance genes are replaced with differently colored fluorescence genes. Gene-corrected cells are isolated by FACS. We tested the method with mouse ES cells having a mutant hypoxanthine phosphoribosyltransferase (Hprt) gene and grown on feeder cells. After 5 days in culture, gene-corrected cells were obtained free from feeder cells at a "purity" of $>30\%$, enriched $>2,000$ -fold and with a recovery of approximately 20%. Corrected cells were also isolated singly for clonal expansion. Our FACS-based procedure should be applicable at small or large scale to stem cells that can be cultured (with feeder cells, if necessary) for ≥ 3 days.

#5. A closer look at high-speed cell sorter yield: Sacrificing yield for speed? *David Leclerc and Ryan Duggan. University of Chicago Flow Cytometry Facility, Chicago, IL.*

The necessity to sort rare populations from large sample sizes has proven to be paramount in today's research arena. The current study focuses on the recovery of cells sorting at such high speeds. Two cell sorters (BD FACSAria and DAKO MoFlo) were used to sort a high frequency population (B cells) and a low frequency population (Cytotoxic T cells) from mouse spleen. The sorters were set up according to the facility's routine settings with regards to pressure, tip size, and frequency of droplet formation, and the volume flow rate was standardized on each instrument. Percent recovery was calculated taking into account the various abort possibilities at high, medium and low throughput rates. This study highlights proper methods for testing recovery from any droplet cell sorting instrument.

#6. Caveolin-1 Knockout Lung Endothelial Cells Demonstrate Abrogated ROS Production and Cell Proliferation with Altered Shear Stress. *Tatyana Milovanova, Muniswamy Madesh, Shampa Chatterjee, Brian Hawkins, Kris De'Bolt, *Jonni S. Moore and Aron B.*

We showed previously that abrupt decrease in flow in the pulmonary microvasculature leads to the production of ROS mediated by NADPH oxidase and that ROS serve as a signal for EC proliferation due the acute loss of shear stress. We evaluated the role of caveolin-1- for –ROS-induced cell proliferation in flow-adapted mice pulmonary microvascular EC in response of cessation of flow. To study proliferation, PKH26 labeled EC were seeded in an artificial capillary system and subjected to continued flow at 5 dynes/cm² for 72 h followed by 24h of stop flow (“ischemia”). ROS production, measured by DCF fluorescence, was increased significantly during the first hour of ischemia in WT. Caveolin-1 phosphorylation was demonstrated during 10-15 min period following flow cessation by Western blot and FACS analysis, and was not affected by inhibitors of ROS production. After 24h of ischemia, there was a 2.5-fold increase in the proliferation index and a 6-fold increase of cells in S+G2/M phases, which was abolished by the inhibitors of ROS production. Flow cessation with caveolin-1 null PMVEC compared to WT resulted in markedly decreased ROS production and EC proliferation with G2/M arrest. These studies indicate that caveolin-1 can function if flow-adapted MPMVEC as a shear sensor resulting in a ROS mediated signaling response following the abrupt reduction in flow. [HL60290].

#7. Effect of vaccination and/or challenge of swine influenza virus on the proportion of activated cells in bronchoalveolar lavage fluid. *Kayoko Kimura, Amy L. Vincent, Juergen A. Richt, Kelly M. Lager, James A. Roth, and Marcus E. Kehrli, Jr. Iowa State University, National Animal Disease Center, USDA/ARS.*

Swine influenza, caused by influenza A virus (SIV), is described as an acute respiratory disease with a high morbidity in pigs. Killed vaccines that contain both H1N1 and H3N2 subtypes are available commercially; however, due to recently emerging novel subtypes and genetic/antigenic variants, effective management of SIV remains a challenge. We have tested whether the use of NS1-truncation mutant, based on the TX98 (H3N2) strain as a modified live vaccine (MLV), might protect pigs from SIV infection with wild type TX98, an antigenically distinct H3N2 (CO99), or the heterosubtypic H1N1 (IA04) isolates. We investigated whether protection was associated with the activation and recruitment of lymphocytes in bronchoalveolar lavage fluid (BALF). Seventy SIV negative pigs (5 weeks old) were divided into 8 groups as shown in Table I. Three weeks after intranasal vaccination with the MLV, pigs were boosted with a second dose of the MLV. Two weeks after the boost, pigs were challenged intratracheally with one of three SIV isolates and euthanized 5 days after challenge. BALF was obtained at necropsy and lymphocyte populations were examined by flow cytometry for the expression of CD44, CD18, CD4, CD8, N4 (TCR-1), IgM, MHC-II, and CD25. Vaccination induced complete protection from challenge with wild type TX98, nearly complete protection from CO99, and partial protection from IA04. Without vaccination, pigs with TX98 challenge had the lowest percentage of pneumonia lesions, followed by CO99. The IA04 challenged pigs had substantially greater percentages of lung involvement. BALF from challenged pigs contained significantly more lymphocytes when compared to the non-challenged controls. In our assays, CD44^{high}CD18^{high}, CD25⁺ cells, and CD4⁺CD8⁺, MHC-II⁺ T-cells are considered “activated”. A lower CD4/CD8 ratio indicated an increase in CD8⁺ cells, which are thought to be important for immune protection of the host from virus infections. The CD4/CD8 ratio was very low in Groups 2 (0.11 +/- 0.05), 3 (0.11 +/- 0.02), and 6 (0.08 +/- 0.04), followed by Groups 1(0.26 +/- 0.02), 7 (0.48 +/-

0.05), 8 (0.69 +/- 0.11), 4 (0.71 +/- 0.07) and then 5 (1.36 +/- 0.16). Vaccination decreased the CD4/CD8 ratio, thus increased the migration of CD8+ cells into the lung. In addition, a pattern of increased CD44^{high}CD18^{high} cell percentage of total lymphocytes was observed in vaccinated pigs. Within the CD8+ lymphocyte population, these percentages were TX98>CO99>>IA04. They increased in MLV-vaccinated, TX98- and CO99-challenged pigs (80.2 +/- 2.0 % and 68.8 +/- 3.9 %, respectively) when compared to non-vaccinated TX98 and CO99 challenged pigs (63.0 +/- 3.7 % and 58.3 +/- 2.8 %, respectively). However, the percentages were nearly equal in IA04 non-vaccinated or vaccinated and challenged pigs (38.0 +/- 4.9 % vs. 35.6 +/- 6.0 %). A similar pattern was observed in other activation marker expressions. However there was no effect of MLV-vaccination on CD44^{high}IgM+ cells. The percentage of activated non-B cells tended to correlate with the degree of protection. These results suggest that the degree of recruitment and activation of lymphocytes, especially CD8+ cells, are associated with the protection from the SIV-induced damage. Virus specific CD8+ cells might be involved in the immune protection induced by the MLV.

Table I.

Group	Vaccination	Challenge	Number of pigs
1	No	None	5
2	Yes	None	5
3	No	Texas 98 (H3N2)	10
4	No	Colorado 99 (H3N2)	10
5	No	Iowa 04 (H1N1)	10
6	Yes	Texas 98 (H3N2)	10
7	Yes	Colorado 99 (H3N2)	10
8	Yes	Iowa 04 (H1N1)	10

#8. Exploiting the illumination volume: An alternative compensation control. *Ryan Duggan and Michael Olson. University of Chicago Flow Cytometry Facility, Chicago, IL.*

The ideal compensation control would be a single stained sample using the actual antibody:fluorochrome pair that is used in the sample, and that is as bright as possible with a tight distribution. Methods used to try and achieve this have included using antibody capture beads, using a surrogate antibody (CD8), or beads that approximate the spectral characteristics of the fluorochrome. Each of these have their short-comings and such an alternative method is presented which attempts to exploit the illumination volume: the volume of fluid that is illuminated by the laser beam when a cell is in its presence.

#9. Automated classification and recognition of bacterial particles in flow by multi-angle scatter measurement and support vector machine classifier. *Bartek Rajwa (1), Murugesan Venkatapathi (1,2), Kathy Ragheb (1), Padmapriya P. Banada (3), E. Daniel Hirleman (2), Todd Lary (4), and J. Paul Robinson (1). (1) Purdue University Cytometry Laboratories, (2) School of Mechanical Engineering, Purdue University, (3) Molecular Food Microbiology Laboratory, Department of Food Science, Purdue University, (4) Cellular Analysis Technology Center, Beckman Coulter, Inc.*

Biological microparticles scatter light in all directions when illuminated. The complex scatter pattern is dependent on particle's size, shape, refraction index, density, and morphology. Commercial flow cytometers allow measurement at two nominal angles ($2^\circ \leq \theta_1 \leq 20^\circ$ and $70^\circ \leq \theta_2 \leq 110^\circ$) of scattered light intensity from individual microparticles with a speed varying from 10 to 10000 particles per second. The choice of angle is

dictated by the fact that scattered light in the small-angle region is primarily dependent on cell size and refractive index, whereas side scatter intensity is dependent on the granularity of cellular structures. Obviously, these rudimentary measurements cannot be used to separate populations of cells of similar shape, size, or structure. Hence, there have been several attempts in flow cytometry to measure the entire scatter patterns. However, the published concepts required use of unique custom-built flow cytometers and could not be applied to existing instruments. It was also not clear how much information about patterns is really necessary to separate various populations of cells present in a given sample.

The presented work demonstrates application of pattern recognition techniques to classify particles on the basis of their discrete scatter patterns collected at just five different angles, and accompanied by the measurement of axial light loss. Our approach can be used with existing instruments and requires only the addition of a compact custom-built scatter-detector. Our analytical model of scatter of laser beams by individual bacterial cells suspended in a fluid was used to determine the location of scatter sensors. Experimental results were used to train the SVM-based pattern recognition system. It has been shown that information provided just by six scatter-related parameters was sufficient to recognize various bacteria with 90-99% success rate.

#10. Study of Molecular Mechanisms of Escherichia coli Response to Monochloramine Using DNA Microarray Technology Combined with Flow

Cytometry. *Diane J. Holder, David Berry, Dongjuan Dai, Lutgarde Raskin, and Chuanwu Xi. Department of Environmental Health Sciences, University of Michigan, and Department of Civil and Environmental Engineering, University of Michigan.*

Resistance to disinfectants may be partially responsible for the higher than expected persistence of certain bacteria in drinking water distribution systems. This resistance could be due to environmental factors, such as exposure to lower than anticipated disinfectant concentrations because of mass transfer resistance when bacteria are present in biofilms.

Initial DNA microarray experiments in this laboratory showed that 58 genes were induced three-fold or more and 51 genes were repressed at least three-fold in *E. coli* K-12 cells exposed to sub-lethal concentrations of monochloramine compared to control cells without monochloramine exposure. Four genes (*ykgB*, *ykgC*, *ykgI*, and *ykgL*) with the highest induction (around 20-fold induction) are in the same region of the *E. coli* K-12 chromosome, and their function is currently unknown. Three of these genes (*ykgB*, *ykgC*, and *ykgI*) are located side by side and are in close proximity to an AraC type transcriptional regulator. AraC type regulators are typically involved in the regulation of efflux-pump systems. Based on sequence homology analysis, *ykgB* appears to encode a transmembrane protein, *ykgI* encodes a putative periplasmic protein, and *ykgC* appears to encode an oxidoreductase with FAD/NAD(P)-binding domain and dimerization domain. The fourth gene, *ykgL*, is in close proximity to the other three and appears to encode a membrane-associated protein. Combined, this information suggests that these genes may be components of a novel efflux mechanism specific for monochloramine, or similar compounds. Each mutant and the wildtype (K12) strain were stained with Invitrogen LIVE/DEAD BacLight Bacterial Viability Kit *for microscopy and quantitative assay. Flow cytometric analysis of the initial effects of exposure to 2 mg/L of monochloramine on *E. coli* mutants lacking these four genes ($\Delta ykgB$, $\Delta ykgC$, $\Delta ykgI$ and $\Delta ykgL$) indicated that while there was little difference in viability between mutants and wildtype. When

stained with propidium iodide, two of the mutants had different patterns of fluorescent dye incorporation, compared to the wild type cells. Propidium iodide, which can only enter cells with damaged cell walls, was incorporated at higher rates in mutants ΔykG B and ΔykG L (60% and 51% of the population respectively, showing strong fluorescence) than in the other mutants (37 % ΔykG C and 25 % ΔykG I) or in the wild type strain (25%), this may further confirm that these genes are involved in protecting cells from toxic compounds.

#11. EFFECTS OF HEAT SHOCK PROTEIN 72 ON NEUTROPHIL FUNCTION.

Andrew Osterburg¹, Sandy Schwemberger¹, George F. Babcock². ¹Shriners Hospitals for Children Research, Cincinnati, Ohio, ²University of Cincinnati, Surgery, College of Medicine, Cincinnati, Ohio.

Heat shock proteins (HSP) play an important role in the regulation of the immune response following severe traumatic injuries, such as burns. We have previously reported that following severe thermal injury, neutrophils (PMNs) display altered function including altered ability of undergo apoptosis and upregulate CD11b. In this study, we examined effect of HSP72 expression on nuclear factor kappa B (NF- κ B), apoptosis, and CD11b in PMNs. HSP72 expression was induced in PMNs obtained from healthy individuals by incubation at 41°C for 60 min. and detected by flow cytometry. Apoptosis is induced by incubating PMNs with 400 IU of TNF α for 90 min. HSP72 expression, CD11b expression, NF- κ B, and apoptosis were detected by flow cytometry following the binding of fluorochrome labeled antibodies to HSP72 (HSP70b'), CD11b, NF- κ B or fluorochrome labeled annexin V for apoptosis. Confocal microscopy studies, and in some cases immunoprecipitation followed by Western blotting, were also performed to detect CD11b and co-localization. Incubation at 41°C induced the expression of HSP72 in nearly 100% of the PMNs. Following treatment with TNF α , 30-45% of PMNs were apoptotic. In PMNs expressing HSP72, apoptosis was reduced to <10%. The levels of the active form of NF- κ B (p65) increased in PMNs expressing HSP70. Immunoprecipitation studies indicated that HSP72 and NF- κ B co-precipitated and, thus, may influence its translocation to the nucleus. Co-localization to the nucleus was demonstrated by confocal microscopy. The reduced up-regulation of the active epitope of CD11b correlated with the level of expression of HSP72. The data is indicative of multiple roles for HSP72, an anti-apoptotic role mediated through NF- κ B, and an alteration in upregulation of the active epitope of CD11b.

#12. Analysis of Signaling Pathways in Acute Leukemia Patients by Flow Cytometry, and Application to Pharmacodynamic Monitoring of Signal

Transduction Inhibitors. *Sue Chow and David Hedley. Princess Margaret Hospital, Toronto, ON.*

The combined use of multiple phosphospecific antibodies plus standard phenotypic markers has recently emerged as a powerful flow cytometry application for studying signaling pathways at the single cell level. Our own interest has focused on the development of applications for pharmacodynamic monitoring of small molecule inhibitors in whole blood samples, linked to early phase clinical trials of small molecule inhibitors. This approach was successfully applied to a phase I clinical trial of BAY 43-9006 in refractory AML patients, conducted by the National Cancer Institute of Canada. The laboratory protocol examined effects of Stem Cell Factor and PMA to activate ERK in the leukemic blast population. Interestingly, we found that during treatment with BAY 43-9006, peripheral blasts became resistant to activation by SCF but not by PMA, suggesting that the drug was inhibiting c-Kit to a greater extent than raf kinase. To examine this further, we determined the IC50 values for BAY 43-9006 to inhibit ERK in

blood samples obtained from peripheral stem cell donors. We found that activation by SCF was inhibited at 5-10mM BAY 43-9006, which is clinically achievable, whereas activation by PMA required >200mM.

We recently introduced several enhancements to our original technique, including small blood volumes, rapidity of sample handling, and ability to detect rare subpopulations in peripheral blood samples. These optimize it for routine clinical application. In addition to ERK, the technique also incorporates measurements of Akt and S6 ribosomal protein phosphorylation, and the ability to modulate these by the addition of growth factors such as SCF and Flt3 ligand, or small molecule inhibitors such as LY294002 and rapamycin. It is thus sensitive to PI3-kinase and mTOR as well as ERK pathway activation. Applying this to a series of 19 AML patient samples, we identified increased constitutive PI3-kinase and mTOR signaling in about half of the cases, and evaluation of the clinical significance of this is ongoing. In summary, we have developed flow cytometry methodology that is able to measure activation of several key signaling elements that are aberrantly regulated in acute leukaemia. An important aspect of this work is that the analytical methods have been optimized for pharmacodynamic monitoring, including the potential for real time monitoring of novel agents.

#13. Measurement of DNA Damage Repair in Molt4 Cells and Normal T Lymphocytes in Response to Gamma Radiation Exposure. *Tammy Stefan, Stanton L. Gerson, James W. Jacobberger. Case Comprehensive Cancer Center, Cleveland, OH*

To ask whether we could determine a difference in repair of double strand breaks in DNA between transformed and normal cells, we measured changes in gamma H2AX immunoreactivity over time following gamma radiation exposure of the T cell lymphoma cell line, Molt4, and cultured, normal human donor T lymphocytes. Measurements were performed by multiparametric flow cytometry that included co-measurement of DNA content and a mitotic marker, p-histone-H3, to provide cell cycle information. Molt4 cells and cultured, stimulated lymphocytes were irradiated with 5 Gy gamma radiation from a cesium source and incubated at 37 deg C for 3 hours. Samples were taken at periodic intervals, and fixed and stored by published methods (Jacobberger et al., *Cytometry* 54:75-88, 2003). Prior to measurement, cells were stained with primary conjugate antibodies and DAPI. Molt4 and T lymphocytes were measured on different days; instrument standardization and sample fluorescence normalization were used to compare data. The cell cycle-related pattern of gamma H2AX expression demonstrated increasing levels of gamma H2AX as a function of the cell cycle, with a distinct and abrupt increase in mitotic cells. Gamma H2AX expression increased to a peak of expression in all phases by 30-40 minutes and then declined in a regular fashion over the next 2.5 hours. Normal lymphocytes and Molt4 cells displayed different kinetics within this overall pattern. There also appeared to be distinct cell cycle differences. Normal lymphocytes showed a delayed onset of gamma H2AX expression. Both cell types appeared to repair (loss of signal) at the same initial rate, but Molt4 appeared to plateau at 3 hours whereas normal cells appeared unchanged. We conclude that there is a difference in the kinetic pattern of gamma H2AX expression between Molt4 and normal T cells.

#14. Measurement of tightly bound PCNA and mcm6 levels provide a cytometric assay of the G1 restriction point (R). *Phyllis S. Frisa and James W. Jacobberger. Case Comprehensive Cancer Center, Cleveland OH*

Cytometric measurement of DNA content and residual mcm6 (mcm6*) after detergent permeabilization demonstrate a bimodal pattern of expression in G1 (Cytometry 68:10-18, 2005). Based on this and the function of mcm6 in replication origin licensing, we tested the hypothesis that high mcm6* G1 cells represented cells that were past the G1 restriction point. We developed a combined assay that included measurement of tightly bound PCNA (PCNA*), DNA content, and in some cases phospho-S10-histone H3, to clearly define the cell cycle.

hTERT-BJ1, hTERT-RPE and Molt4 cells were extracted with Triton X-100 and then fixed in methanol, then stained with primary conjugated antibodies by published methods (Cytometry 7:356-64) for cytometry. Plots of mcm6, PCNA and DNA revealed a complex pattern with distinct clusters. Kinetic experiments, employing serum withdrawal and release and the mitotic inhibitor, nocodazole, or the S phase inhibitor, aphidicolin demonstrated that mcm6* cells cycled in unison with the committed phases of the cell cycle (S, G2, and M). This supports our hypothesis, and a multiparametric assay of mcm6, PCNA, and DNA content in detergent-extracted cells provides a measurement of pre and post-restriction point cells.

#15. In-vitro Measurement of Smooth Muscle Cell Proliferation and Inhibition by Combination of Growth Factors (VEGF, PIGF and FGF-2) In Porcine Direct Coculture Using Flow Cytometry. *Supriya Mocherla and Michael H Peters. Virginia Commonwealth University*

Intimal hyperplasia(IH) is the major drawback of many vascular interventions. The objective of this study is to look at the role of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2) as therapeutic agents to IH. VEGF and FGF-2 were recognized as potential mitogens and chemo attractants for endothelial cells (EC's), and inhibitors of smooth muscle cell (SMC) proliferation. In this study, a direct in-vitro co-culture experiment was developed to measure both total cell counts and cell cycle behavior as a function of concentration using flow cytometry. Live cell markers, CMFDA and DiI-Ac-LDL, enabled us to identify both SMC and EC, respectively, using fluorescent microscopy. Intracellular staining of anti- alpha smooth muscle cell actin was used to specifically identify smooth muscle cells from the co-culture cells. Cellular DNA was stained with propidium iodide to quantify the cell cycle phases using flow cytometry. Co-cultures were treated with various concentrations of VEGF (0-100ng/ml) for a period of 7-days. VEGF has a no significant effect at inhibiting SMC proliferation. FGF-2 has shown dual role by bringing down the number of cells reaching the cell-cycle synthesis phase at higher concentrations(>10ng/ml). It was also observed that VEGF did not induce apoptosis in either SMC or EC. This study demonstrates the critical role of EC-SMC interactions that lead to SMC proliferation and the clinical occurrence of IH. The endothelial nitric oxide synthase(eNOS) pathway present in EC's has been implicated in previous studies on the control of SMC proliferation. FGF-2 is believed to function as an agonist for the EC-eNOS system resulting in the EC release of vasodilator NO that, in turn, inhibits SMC proliferation.

#16. EFFECTS OF HEAT SHOCK PROTEIN 72 ON NEUTROPHIL FUNCTION. *Andrew Osterburg¹, Sandy Schwemberger¹, George F. Babcock². ¹Shriners Hospitals for Children, Research, Cincinnati, Ohio; ²University of Cincinnati, Surgery, College of Medicine, Cincinnati, Ohio*

Heat shock proteins (HSP) play an important role in the regulation of the immune response following severe traumatic injuries, such as burns. We have previously reported that following severe thermal injury, neutrophils (PMNs) display altered function

including altered ability of undergo apoptosis and upregulate CD11b. In this study, we examined effect of HSP72 expression on nuclear factor kappa B (NF- κ B), apoptosis, and CD11b in PMNs. HSP72 expression was induced in PMNs obtained from healthy individuals by incubation at 41°C for 60 min. and detected by flow cytometry. Apoptosis is induced by incubating PMNs with 400 IU of TNF α for 90 min. HSP72 expression, CD11b expression, NF- κ B, and apoptosis were detected by flow cytometry following the binding of fluorochrome labeled antibodies to HSP72 (HSP70b'), CD11b, NF- κ B or fluorochrome labeled annexin V for apoptosis. Confocal microscopy studies, and in some cases immunoprecipitation followed by Western blotting, were also performed to detect CD11b and co-localization. Incubation at 41°C induced the expression of HSP72 in nearly 100% of the PMNs. Following treatment with TNF α , 30-45% of PMNs were apoptotic. In PMNs expressing HSP72, apoptosis was reduced to <10%. The levels of the active form of NF- κ B (p65) increased in PMNs expressing HSP70. Immunoprecipitation studies indicated that HSP72 and NF- κ B co-precipitated and, thus, may influence its translocation to the nucleus. Co-localization to the nucleus was demonstrated by confocal microscopy. The reduced up-regulation of the active epitope of CD11b correlated with the level of expression of HSP72. The data is indicative of multiple roles for HSP72, an anti-apoptotic role mediated through NF- κ B, and an alteration in upregulation of the active epitope of CD11b.

#17. A Quality Control Program for Digital and Analog Flow Cytometers in a Shared Environment. *Kathryn A. Calmus, William J. Murphy, Charles H. Pletcher, Jr., Jonni S. Moore. Abramson Cancer Center Flow Cytometry and Cell Sorting Resource Laboratory, Path BioResource, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA*

Background and Goal: Daily quality control is an important function in the upkeep and maintenance of instrumentation in a flow cytometry resource facility. Our primary responsibility is to ensure the integrity and accuracy of the data for all investigators who use our facility. As a shared resource laboratory, we must devise procedures and protocols to maintain instrument performance. These protocols must adhere to an established laboratory standard, yet be practical to perform readily in a busy laboratory.

Approaches to instrument quality control are many and varied and no uniform standard exists. Traditionally, each investigator or laboratory develops procedures based upon their specific needs. Attempts at quality control protocol standardization have been suggested (Chase and Hoffman, 1998) and discussed at numerous national and regional flow cytometry meetings. To date, the diversity of protocols remains vast.

Method and Results: We devised a practical quality control program for both our digital (BD LSR II and FACSCanto) and analog (FACSCalibur, FACSort and FACScan) systems. Our first priority was to find a bead standard that excited from UV (355nm) to the red (633nm) and emitted across a wide range of filter sets. We chose a variation using the Sphero 8-Peak Rainbow Calibration Particles, focusing on the 3rd, 5th and 7th peaks (Negative, Dim and Bright bead cocktail, Cat # RCP-30-5-357). We then separated the quality control protocol into four categories: Daily, Weekly, Monthly and Quarterly. Daily quality control consists of running the beads and plotting the median of the brightest peak to detect parameter variation. Weekly quality control focuses on subtle drifts in the cytometers by adjusting laser delays and area scaling using the brightest peak. Monthly quality control focuses on linearity. The beads are acquired at different voltages for each detector, allowing correlation of the dim and bright peak. Quarterly quality control is two-fold. First, optimal PMT voltages are checked by plotting changes

in CV versus changes in PMT voltage. This procedure determines the best signal to background noise using the negative peak. Second, to confirm we have chosen the correct voltage, measurements are made using the negative and bright peaks at 25-volt increments, 100 volts above and below the optimal PMT voltage.

Our approach has proven to be a practical and effective way to provide a standard approach to instrument quality control in a shared environment.

#18. Selective Role of PKC-theta in T Regulatory Cell Development and Function.

Sonal Gupta, Karen Hagen, Zuoming Sun. Department of Microbiology & Immunology, University of Illinois-Chicago; Research Resource Center, University of Illinois-Chicago

T lymphocyte activation and proliferation are the key events in adaptive immune response against pathogens and tumor cell antigens. Equally important to body's well being is the homeostasis of T lymphocytes. Regulatory T cells (Tregs) are critical for maintaining T cell homeostasis by controlling T cell proliferation and autoimmune response against self-antigens. Earlier, it was shown in our lab that Protein Kinase C-theta, which is mainly expressed in T lymphocytes, is required for T cell activation, proliferation and survival after TCR stimulation and CD28 co-stimulation. Since, TCR and CD28 signaling are important for regulatory T cell function and development, we hypothesized that PKC-theta is required for the generation of natural regulatory T cells in the thymus. Differences of CD4 and CD25 surface staining and Foxp3 intracellular staining between thymocytes from PKC-theta-deficient mice and normal control mice appear to support our hypothesis. In addition, we observed a drastic reduction in the numbers of Tregs from spleen and lymph nodes of PKC-theta-deficient mice. To analyze the function of PKC-theta-deficient-Tregs cells, we sorted the CD4+CD25- (responders) and CD4+CD25+ (suppressors) T cells from WT and PKC-theta-deficient mice with the help of AutoMACS (magnetic beads) and MoFlo high speed sorter and performed an in vitro suppression assay. Our data suggested that PKC-theta is dispensable for Tregs in suppression of T cell proliferation, in vitro. We are currently analyzing the function of PKC-theta-deficient Tregs, in vivo.

#19. Multi-parameter flow cytometric deconstruction of humoral responses. *RA*

Barrington, K Ketman, N Barteneva. The CBR Institute for Biomedical Research and Department of Pathology, Harvard Medical School, Boston, MA

Measurement of antibody production is a critical feature in vaccine design. However, the production and persistence of memory B cells may be more informative for long-term humoral protection against pathogens. To determine how the production and persistence of memory B cells changes with time after encountering pathogens, we have utilized 7-8-parameter polychromatic flow cytometry to deconstruct B cell subpopulations during ongoing immune responses to a model protein antigen. Flow cytometry analysis and cell sorting was performed on a 3-laser FACSAria system. The compensation values were established using single colors and FMO (fluorescence minus one) controls and Diva acquisition software. Acquired data was subsequently analyzed using FlowJo (Treestar, Ashland, OR).

During the active response, we can now subdivide the B cell response into those being selected versus those being eliminated. Further, within the responding B cell pool, we find multiple B cell receptor usage. After the initial response, we find evidence of memory B cells persisting during the subsequent 4-5 months following antigen encounter. This preliminary work suggests it will be of value in the evaluation of experimental vaccines.

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