

GLIIFCA

SCIENTIFIC AGENDA SIXTH ANNUAL MEETING

October 3, 4 and 5, 1997

BEST WESTERN/MIDWAY -AIRPORT HOTEL, MILWAUKEE, WISCONSIN

Site Organizer: Alex Nakeff

Local Host: Lynn Peterson

Program Chairs: Mo O’Gorman, Ph.D., Chuck Goolsby, Ph.D.

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FLOWDOWN AND RECEPTION

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PROGRAM

Friday Evening October 3

- 8:00-9:00 PM Guest Lecture: Microbiology Applications of Flow Cytometry-
Steven M. Callister, Gundersen/Lutheran Medical Center,
Symposium Room
- 9:00-11:00 PM **Reception**, Open Bar 9 - 11 PM (Aviator Rm.-Bar /
hors d'oeuvres- Concourse Rm)
Sponsored by: BDIS/PharMingen, Inc.

Saturday Morning, October 4

7:00 - 8:00 AM Continental Breakfast in Aviator Room

Plenary Session I - (Symposium Room)
**Clinical Sciences - Applications and Functional Analyses in
Immunology and Microbiology chair: Maurice R. O’Gorman**

- 8:00 - 8:05 Welcoming Remarks, **Carl Stewart, Lynn Peterson**
- 8:05 - 8:45 “Fluorescence Quantitation” -**Sujata Iyer**
- 8:50 - 9:35 “Flow Cytometric Evaluation of Lymphocyte Activation and
Proliferation”- **Thomas M. Ellis**
- 9:40 - 10:25 “Flow Cytometric Evaluation of NK Function”-
Alice Gilman-Sachs
- 10:30 - 11:20 “**Coffee and Attended Poster Session A** (Please go to the
Concourse Room area where competing authors with posters
designated with A will stand at their respective posters to
present their work)

Platform Presentations

- 11:20 - 11:40 “Effect of Diet on the Immune Response” - **Susan E. Frede**
- 11:40 - 12:00 “CD69 Expression in Renal Transplantation” - **Jimmy Loo**
- Lunch Aviator Room 12:00 - 1:00 (see info sheet)

Saturday Afternoon, October 4

Plenary Session II - (Symposium Room)

BASIC SCIENCE I -Cell Proliferation, Cell Death and Tumor Progression chair: *Charles Goolsby*

- 1:00 - 1:45 "DNA Ploidy and Cell Cycle Analyses- The Future" - **Charles Hitchcock**
- 1:50 - 2:35 "The Role of the Bcl-2 Family of Oncogenic Proteins In Apoptosis" - **Frank Mortari**
- 2:40 - 3:25 "Modulation Of Fibroblast G1 By TGF- β 1 Uses The Cyclin D-Rb Pathway" - **James Jacobberger**
- 3:30 - 4:15 **Coffee and Attended Poster Session B** (Please go to the Concourse Room where competing authors with posters designated with B will stand at their respective posters to present their work)

Platform Presentation

- 4:15 - 4:40 "Post Stress Neutrophil Survival and Apoptosis" - **Joseph G. Meyer**
- 5:00 - 6:30 **Wine and Cheese Happy Hour (and a half)** open bar Aviator Rm./Concourse Rm. **Sponsored by Spherotech, Inc.**
- 5:30 - 6:30** **Poster Viewing: All authors will attend their posters.**
- 7:30 - 12 AM **"ROLL WITH THE FLOW " FLOWDOWN- Aviator Rm. Sponsored by BDIS/PharMingen, Inc.**

Sunday - October 5

Plenary Session III - (Symposium Room)

BASIC SCIENCE II -Future Directions in Clinical Cytometry and Image Analysis, chairs: *Mo O’Gorman and Chuck Goolsby*

- 7:30 - 8:30 Continental Breakfast in Aviator Room
- 8:30 - 9:00 “Evaluation of the T-cell Repertoire Using Antibodies Directed Against the TCR V β Families” - **Roy Overton**
- 9:00 - 9:30 “True Multiplexed Analysis Using the FlowMetrix System” - **Ralph McDade**
- 9:30 - 10:00 **Coffee and Attended Poster Session C** (Please go to the Concourse Room where competing authors with posters designated with C will stand at their respective posters to present their work)
- 10:00 - 10:45 “Flow Cytometric Analysis of Minimum Residual Disease “- **Carl Stewart**
- 10:50 - 11:35 “Functional Analyses of Early Events In Apoptosis”- **David Hedley**
- 11:40 - 12:00 Wrap up and Award Presentations

GLIIFCA SIX

ABSTRACTS

Lymphoproliferative Disorder In CTLA4 Knockout Mice Is A TH2 Mediated Phenomenon That Is Blocked By Treatment With CTLA4Ig

J.A. Auger, R. Khattri, A.H. Sharpe and J.A. Bluestone, *Brigham and Women's Hosp, Harvard Medical School, Boston, MA and The University of Chicago, Chicago, IL.*

Mice lacking CTLA4 die at an age of 2-3 weeks due to massive lymphoproliferation, leading to lymphocytic infiltration and destruction of major organs. The onset of the lymphoproliferative disease can be delayed by treatment with CTLA4Ig which blocks CD28/B7 interactions starting as late as day 12 after birth. The T cells present in CTLA4^{-/-} mice express activation markers, proliferate spontaneously *in vitro* and cannot be costimulated with CD28. In addition, these T cells secrete extremely high levels of IL-4 upon TCR activation. After CTLA4Ig treatment, the activation phenotype is absent and cytokine production is not skewed towards a TH2 cytokine profile. Although the T cells from CTLA4^{-/-} mice cannot be co-stimulated with CD28 they survive longer in culture possibly due to higher expression of survival factors such as bcl-x_L. Thus, it appears that there is peripheral activation of T cells soon after birth that leads to a massive increase in apoptosis resistant, TH2-like CD4 T cells which cause the autoimmune disease in a CTLA4^{-/-} mice. The activation is dependent on CD28/B7 interaction as early treatment with CTLA4Ig blocked the early activation and lymphoproliferation and delayed the onset of disease by 5-6 weeks.

Effect of Diet on the Immune Response

*Susan E. Frede, James P. Cornelius, J.W. Alexander, G. F. Babcock
Shriners Burns Institute and University of Cincinnati
Cincinnati, OH 45229*

Previously we have reported that rat cardiac allograft recipients receiving a diet high in arginine and low in ω 6 fatty acids displayed a shift toward TH2-type cytokines and increased graft survival. In this study human kidney transplant patients were given dietary supplements (lipid (low ω 6)/high arginine or high arginine) for 18 days followed by MLR and cytokine analyses. Intracellular levels of IL-4 and IFN- γ produced by CD3⁺CD4⁺ and CD3⁺CD8⁺ cells were measured by flow cytometry. We found that patients receiving arginine alone showed an overall increase (~2X) in the percentage of cell producing all cytokines measured. Those patients receiving lipid/high arginine displayed a slight increase in IFN- γ (1X - 2X) and a marked increase in IL-4 (3X - 6X) production. These results suggest that arginine alone may stimulate cytokine production, but increased lipids (low ω 6) are needed to get the shift toward IL-4, which has been shown in our lab and in recent literature to be associated with tolerance.

Functional Analyses Of Early Events In Apoptosis

*David W. Hedley
Ontario Cancer Institute
Toronto, Ontario, Canada M5G 2M9*

Apoptosis is an active process, characterized by a series of biochemical changes that are regulated by genes such as the bcl-2 family, c-myc, and p53. These changes culminate in an "executioner" phase; e.g. activation of caspases or calcium-dependent endonucleases. However, it is not yet understood how the various players interact to regulate cell death *in vivo*. Failure to undergo apoptosis following treatment with DNA damaging agents is a major cause of treatment failure in cancer patients. Our laboratory has approached this problem by developing multiparameter techniques for studying biochemical changes during drug-induced apoptosis. These methods are based on viable cell probes for calcium, intracellular pH, glutathione, reactive oxygen, and mitochondrial membrane potential. Typically they employ 3-4 probes per sample, with 2-3 laser excitation and 5 PMT's. They provide a unique insight into the sequences of events that occur during apoptosis, and identify the sites where genes such as bcl-2 act to inhibit cell death. In combination with standard molecular biology techniques, this is likely to prove a powerful

approach for understanding how cancer cells evade cell death following chemotherapy, and for pinpointing potential targets for novel treatment strategies.

Let Me Count the Ways

Sujata Iyer, Ph.D.

Reagent Development Project Manager and Program Manager Quantitation

Becton Dickinson Immunocytometry Systems

San Jose, CA

The number of PE-conjugated antibodies bound to a cell can be quantitated on a flow cytometer with PE beads of known copy number. However, a number of factors affect the accuracy of quantitation and conclusions about epitope density. These factors include: valence of antibody binding: the use of Fab's vs intact mAbs; fixation; the purity of the conjugate (i.e. % 1:1s); off rate; location of epitope on target molecule; washed vs unwashed preparations; and conjugate stability.

The QuantiBRITE™ consists of QuantiBRITE PE beads, Quantiquest the quantitative calibration feature in CellQuest and analysis software. The performance of the QuantiBRITE system will be discussed, using CD4 as a model.

CD69 Expression In Renal Transplantation

J. Loo and P.A. Keown

Immunology Lab., VGH

Vancouver, B.C. Canada

In this study, we examined the expression of the T-cell activation marker CD69 as an index of the immune status following renal transplantation and a potential graft rejection indicator. Peripheral blood mononuclear cells were activated *in vitro* with mitogens and CD69 expression was measured by flow cytometry. In CD4+ve T-cell subset, its level increased rapidly to peak by 24 hours ($0.2 \pm 10\%$ to $26.0 \pm 2.2\%$; $p < 0.001$), and returned to baseline by 72 hours. Cyclosporin A ($13.3 \pm 3.8\%$), FK506 ($20.0 \pm 2.7\%$) and methylprednisolone ($18.3 \pm 2.1\%$) reduced and delayed, but did not prevent, expression *in vitro*. CD69 levels in hemo- ($31.1 \pm 5.3\%$) or peritoneal dialysis ($19.3 \pm 3.0\%$) patients did not differ from normal controls. Expression was markedly decreased within the first month post-transplant ($4.5 \pm 3.1\%$) and returned to normal in stable patients 1 year or more post-transplant ($21.0 \pm 3.4\%$). Conclusion: CD69 is a robust marker of T-cell activation which is suppressed by intensive immunosuppression *in vivo*.

Post Stress Neutrophil Survival and Apoptosis

Joseph G. Meyer and G. F. Babcock

Shriners Burns Institute and University of Cincinnati

Cincinnati, OH 45229

Our laboratory has previously demonstrated that following severe thermal injury or trauma, peripheral blood neutrophils (PMNs) displayed dysfunction. The observed defects included: suppressed phagocytosis of bacteria oxidative metabolism and upregulation of CD11b following treatment with LPS. In this study an *in vitro* model of severe stress, heat shock, was used followed by an examination of apoptosis and expression of heat shock proteins (HSP72). PMA and LPS were used as inducers of apoptosis and inflammatory stimuli respectively. Apoptosis was measured by flow cytometry using Annexin V binding in conjunction with 7-amino-actinomycin D (7-AAD). We concluded that heating cells *in vitro* at 43°C resulted in the induction of HSP72 in nearly all PMNs. Also, the presence of HSP72 in these cells directly correlated with dysfunction. In addition, treatment with LPS or the presence of HSP72 enhanced the resistance of PMNs to the induction of apoptosis by phorbol esters. These results suggest that LPS not only activates normal PMNs but may actually enhance the survival of PMNs following severe stress.

The Role Of The Bcl-2 Family Of Oncogenic Proteins In Apoptosis

Frank Mortari, Ph.D.

R & D Systems

Minneapolis, MN 55413

The health of the host is maintained through a fine balance in the cellular composition and content of any organ. This cellular homeostasis is largely controlled through a process referred to as apoptotic cellular death. The initial discovery of the Bcl-2 oncogene has given rise to a vast body of evidence that suggests that this protein plays an important regulatory function in determining whether a cell lives or dies. In the last decade, a number of Bcl-2 related genes and proteins have been described that interact with each other. This family of related proteins have both pro-apoptotic and anti-apoptotic functions. The net result of these intracellular protein interactions is to deliver either a survival or a death signal to the cell.

The ability of a cell to respond or not to respond to an apoptotic signal has been associated with a variety of pathologies, such as: tumor progression, autoimmune disease, developmental abnormalities and progressive viral infections. To better understand the complexities of this cell death program, it is necessary to understand the proteins that regulate this process. This will be a review of the key players within the Bcl-2 family of proteins and how their activities appears to be determined by their phosphorylation state and/or their association status with other proteins found within the cytosol. The goal is to solicit ideas to develop reagents and assays which can be of diagnostic value that can monitor these regulatory cell death protein structures.

EVALUATION OF CD40 LIGAND (CD154) EXPRESSION ON NEONATAL CORD BLOOD T CELLS USING A RAPID WHOLE BLOOD FLOW CYTOMETRY PROCEDURE.

M.Paniagua, D. Zaas, M.R.G. O'Gorman.

Northwestern University Medical School, and The Children's Memorial Hospital, Chicago, IL

Our objective was to establish the level of expression of the CD40 Ligand (CD154) on resting and *in vitro* activated cord blood T cells using a three color, whole blood flow cytometry method developed in our laboratory as a screen for the X-Linked Hyper IgM Syndrome (XHIM). Several labs have reported significantly diminished percentages of CD154 on *in vitro* activated neonatal cord blood T cells as compared to healthy adult (HA) T cells; this would suggest that cord blood would be insensitive for the detection of abnormalities in CD154 expression, and therefore a poor screen for XHIM. Our evaluation of 14 cord blood and 8 HA samples showed that the percentage of CD4+ T cells expressing CD154 was equivalent in both groups (93.5% and 93.5%), however the fluorescence intensity (mfc) of CD154 on CD3+CD8- T cells was slightly but significantly decreased in the cord blood samples. These data indicate this flow cytometry assay provides a sensitive tool for the detection of abnormalities in the expression of CD154 in cord blood and may therefore serve as an effective screen for XHIM.

Flow Cytometric Evaluation of Lymphocyte Activation and Proliferation

Thomas M. Ellis, Ph.D.

Loyola University School of Medicine

Maywood, IL

Techniques for assessing T lymphocyte activation comprise fundamental tools for research immunology as well as for clinical determinations of histocompatibility, immunocompetence and pathologic conditions associated with in situ immune activation. Flow cytometry offers numerous advantages over standard techniques for assessing lymphocyte activation, the most significant of which is arguably its multiparametric capabilities. Lymphocyte activation is accompanied by numerous cellular changes that are readily measurable using flow cytometry, including activation of second messenger pathways, expression of activation cell surface markers, induction of cellular proliferation and functional differentiation. The earliest detectable events (≤ 1 h) following receptor engagement involve the activation of second messenger pathways, leading to increased intracellular calcium concentrations and protein tyrosine phosphorylation. The earliest (≤ 1 h) detectable cell surface activation marker on T cells is CD69, a member of the C-type lectin family, whose expression is first detectable around 1 h of activation and peaks at 20-40 h post activation, depending on the nature of the stimulus. CD69 expression correlates closely with tritiated thymidine incorporation and thus may serve as a useful early index of in vitro activation, although cells other than those directly triggered by antigen or mitogen can also express this marker. Other surface markers appear later (≥ 12 h) and may be more practical for assessing chronic activation; these include CD25, HLDR, and CD71. More recently, the refinement of reagents and techniques for detecting cytoplasmic cytokines has offered a powerful tool for assessing functional consequences of activation at the single cell level. However, one drawback of these techniques remains their limited utility for detecting activated cells in peripheral blood, a problem that might reflect the normal absence of activated cells in the circulation. Nevertheless, flow cytometry supports a host of rapid techniques that not only offer suitable substitutes for current in vitro methods for measuring activation but also significantly expand our capability to characterize the functional consequences of such activation.

Flow Cytometric Evaluation of NK Function

Alice Gilman-Sachs, Ph.D.

FUHS/ Chicago Medical School

Chicago, IL

Natural killer cells are a subpopulation of lymphocytes that have cytotoxic activity against tumor cells. They may also be important in pregnancy; 50% of the lymphocytes in the placenta are CD56+ NK cells. Recently we have set up and evaluated a flow cytometric assay to measure NK functional activity in women with recurrent spontaneous abortions. The assay consists of incubating membrane labeled target K562 cells (stained green with PKH-2 dye) with lymphocytes at E:T ratios of 50:1, 25:1 and 12.5:1. After two hours of incubation, propidium iodide is added which binds to DNA of dead K562 target cells. Flow cytometric analysis of dead target cells is used to determine cytotoxic activity at each E:T ratio. Advantages of this assay are that it is faster than the chromium release assay (since it requires a shorter incubation time) and does not require storage and disposal of radioactive waste. Topics to be discussed at the presentation are choices of target cell labels, flow cytometers, protocols and storage of clinical specimens.

DNA Ploidy and Cell Cycle Analyses - The Future

Charles L. Hitchcock, M.D., Ph.D.

Department of Pathology

The Ohio State University,

Columbus, OH 43210

The future for DNA flow cytometry has drastically changed in the last two-to-three years by a lack of standardized technology and perceived clinical utility. Whereas immunophenotyping is considered a reproducible clinical test and therefore clinically relevant, DNA ploidy and cell cycle analyses are not. Why? We can improve our ability to detect DNA aneuploid cell populations simply changing to a multiparameter approach. We routinely use DNA vs side scatter or FALS vs side scatter to define DNA aneuploid cells in our samples. However, one cannot use this approach for cell cycle analysis. One can use a dual color analyses with FITC-labeled antibodies to cytokeratin and PI staining as used for analysis of urine/bladder washing specimens, fresh samples and even paraffin section of carcinomas. In cases where the sample is too small we can use image analysis or the laser scanning cytometer, both of which allow one to directly select the cells of interest for measurement. No matter the instrumentation or parameters, strict adherence to established interpretive criteria is needed! A lack of standardized technology calls into question the clinical utility of cell cycle analyses. The lack of technologic standardization one must use their own data and not the literature's for assessing cutpoints in you laboratory. What is the best cutpoint? Are their separate ones for DNA diploid and DNA aneuploid? Do you change their values every time you change your techniques and/or analysis programs? The future for DNA flow cytometry is being plagued by the "Show-Me-The-Money" mantra of our hospital's administrators and taht of managed care. In today's clinical environment, if test results leack immediate clinical utility they will not be paid for. No money, no test; no test, no job. The future of DNA flow cytometry is establishment of strict standards and well designed multicenter prospective studies.

Modulation Of Fibroblast G1 By TGF- β 1 Uses The Cyclin D-Rb Pathway

J. W. Jacobberger & D. Zhang,

Cancer Research Center

Case Western Reserve University

Cleveland, OH

Cell cycle transit time is thought to vary largely through regulation of the G1 phase time while S, G2 and M remain relatively invariant. Part of the timing mechanism of G1 may depend on phase transition catalyzed by cyclin dependent kinases at critical active concentrations. These reactions and others that take place between phase transitions may constitute a web of interacting pathways that regulate forward movement by feedback inhibition and stimulation and competing negative and positive reactions. We have previously shown that SV40 T antigen (Tag) and serum are rate-limiting for G1 time of NIH 3T3 cells. This occurred through inhibition of cell density-dependent negative G1 regulation, but a density independent component was also observed. In an attempt to determine the molecular basis for these two modalities, we turned to purified growth factors as simpler modulators of G1 time. TGF- β 1 was perhaps the most interesting of the growth factors we tested. Modulation of fibroblast G1 time by this cytokine was shown to be both density-dependent and -independent. TGF- β 1 regulates G1 time in distinct phases. The first phase, 6 hours after TGF- β 1 treatment of NIH-3T3 cells, is inhibitory. The second phase, at 12 hours, is stimulatory. Both of these modulations appear to be density independent. At 24 hours post treatment, the stimulatory phase is maintained but is by then density dependent. To determine some of the molecular players in this modulation of G1, we have utilized genetic intervention by retroviral transduction of genes coding for molecules that might be involved. Vectors encoding cyclin D1, E2F-1, Tag, and Tag402DN (does not bind p53) showed some ability to abrogate the inhibitory phase. TagK1 (does not bind Rb), cyclin E, and cyclin A had no effect. Kinetic analysis showed that after TGF- β 1 treatment, relative to control cells, cyclin D1 levels decreased and Rb shifted to the hypophosphorylated form prior to lengthening of G1. Thus, it is suggested that TGF- β 1 modulates G1 time, in part, by modulating the cyclin D pathway. At present, we have not been able to genetically abrogate the density independent or dependent stimulatory phase, nor has kinetic analysis by western blotting indicated any molecules as candidates for intervention.

True Multiplexed Analysis Using the FlowMetrix™ System

*Ralph McDade, Kerry Oliver and Jerry Fulton
Luminex Corporation
Austin, TX 78727*

The FlowMetrix System is a flow cytometer based analysis platform that performs up to 64 assays simultaneously. Distinct sets of fluorescent microspheres are modified with assay targets such as antigens, antibodies or oligonucleotides and mixed to form a multiplexed assay set. Reactions are developed using green fluorescent reporters to specifically quantitate each individual assay of the multiples. Assays are read in a standard flow cytometer interfaced with digital signal-processing hardware and software that perform real-time data acquisition and analysis. Multiplexed enzymatic, immunoassay and nucleic acid hybridization analyses have been developed. Benefits include speed, economy and advanced capabilities. This presentation will introduce the concepts of the technology with the results of a multiplexed cytokine panel assay.

Altered Cytokine Production By T Cells From Patients With B-CLL.

*J.S. Moore, M.Zaki, R.Douglas, P.Nowell.
University of Pennsylvania School of Medicine, Philadelphia, PA.*

Chronic lymphocytic leukemia is the most common leukemia in the western world characterized accumulation of CD5+ B cells. Prolonged disease course combined with lack of consistent cytogenetic defects are suggestive of abnormal cytokine production and/or response related to a failure of apoptosis in B-CLL. We suggest that alterations in T cell subsets have a role in regulating apoptosis in CLL through their cytokine production. In a study of 10 untreated CLL patients and 5 normals, we showed that levels of IL-2 & IL-4-producing T cells in B-CLL patients were similar to normals while IFN γ -producing T cells were highly elevated. Interestingly, IFN γ is highly protective of CLL B cells from undergoing apoptosis, thus the increase in percentage of IFN γ -producing T cells could have a significant impact on prolonged survival of the neoplastic clone. We conclude that measurements of cytokine production by T cells may yield insight into the pathogenesis of CLL and offer targets for therapy.

Evaluation of the T-Cell Repertoire Using Antibodies Directed Against the TCR V β Families

*J. Philip McCoy, Jr. and W. Roy Overton
Cooper Hospital/UMC
UMDNJ - Robert Wood Johnson Medical School at Camden
Camden, NJ 08103*

The antigenic specificity of most peripheral blood T lymphocytes is conferred by combinations of V β and variable chains within the T cell receptor complex. Approximately 25 families of V β chains have been described in the human. Examination of the V β chains will lead to a better understanding of the immune response, and appears to be useful in many clinical situations. While genes encoding V β chains may be studied using a variety of molecular techniques, flow cytometric studies of these structures offer 1) the ability to examine functional proteins, rather than encoding genes; 2) the opportunity to perform multiparameter studies, identifying V β chains on specific T cell subsets; and 3) easy enumeration of the number of cells bearing specified V β chains.

In the study of mature T cell leukemias and lymphomas (those expressing CD3), analysis of V β chains may be used as an indicator of clonality, similar to how immunoglobulin light chain analyses are conducted in mature B cell neoplasms. The task is considerably more complicated with T cells, as 25 V β chain families must be examined, rather than 2 light chains as with B cells. The study of V β chains in HIV disease is also of interest from both the basic research and clinical perspectives. Retroviruses encode "superantigens" which circumvent typical T cell recognition by relying solely on V β chains rather than V α -V β chain complexes. This has spawned a great deal of interest in the role of V β chains in HIV disease. Key questions include whether CD4 T cell depletion in HIV disease is random or is related to V β chain expression by CD4 cells; whether CD8 cells respond to HIV infection based on V β chain expression; and if CD4 recovery following therapy with protease inhibitors is clonal or polyclonal.

Limitations still exist to the routine use of antibodies to V β chains in clinical studies. Chief among these is the fact that antibodies to all V β chain families are not yet available, thus the complete repertoire cannot be identified. Secondly, these reagents represent a costly and cumbersome panel to analyze routinely. Whether these reagents find their way into routine clinical use remains problematic. Nonetheless, flow cytometric analysis of TCR V β chains remains a promising manner in which to study T lymphocyte responses.

Flow Cytometric Analysis Of Minimum Residual Disease

*Carleton C. Stewart, Ph.D.
Director, Laboratory of Flow Cytometry
Roswell Park Cancer Institute
Buffalo, NY 14263*

The ability to monitor patients with leukemia during therapy by flow cytometry may have an impact in producing a cure. To detect residual disease three assumptions are made. The first is that the leukemic cells exhibit aberrant antigen expression allowing them to be distinguished from normal cells. The second assumes that residual leukemic cells have the same immunophenotype on relapse as they had in the diagnostic specimen. The third is all these assumptions require validation. Our findings show that, in AML, small normal populations exist that significantly overlap the apparent aberrant expression, thereby reducing the specificity and sensitivity for detecting residual cells. In all cases the relapse leukemia does not exactly exhibit the same immunophenotype as the diagnostic specimen. The degree to which it is different will effect the sensitivity for detection. The single most important technical problem with residual disease monitoring is variation in staining intensity that occurs when different batches of the same monoclonal antibodies are used. This problem was solved by a data analysis program that could normalize files to a standard reference generic bone marrow file. In so doing, normal target cell populations are located in the same multidimensional space. Our results support the conclusion that patients whose leukemia does not fall below detectable levels during therapy will relapse.