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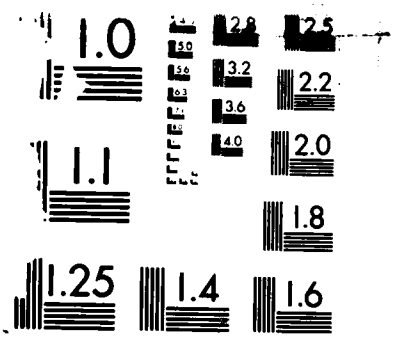

RESOLUTION TEST CHART

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Final Report

BECTON-DICKSON MODEL 420 FLUORESCENCE-ACTIVATED CELL SORTER (FACS)

Richard G. Olsen
Department of Veterinary Pathobiology

For the Period
December 21, 1984 - December 20, 1985

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
Bolling Air Force Base
Washington, D.C. 20332-6448

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The instrument purchased on the Air Force Office of Scientific Research grant 85-0086 was an Epics 741 Single Laser System Flow Cytometer from the Epics Division of the Coulter Corporation, Hialeah, Flordia. The Epics 741 flow cytometer has and is being used in DOD projects entitled "Immune Dysfunctions and Abrogation of the Inflammatory Response by Environmental Chemicals" (AFOSR contract no. F49629-79-C-0163 and AFOSR grant no. #86-0129). The flow cytometry instrument is being used to evaluate the in vitro effects of unsymmetrical dimethyl hydrazine on the distribution and expression of T-lymphocyte subset antigens and Ia antigens.

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For the Period
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AIR FORCE OFFICE OF SCIENTIFIC RESEARCH (AFOSR/PK)
Bolling Air Force Base
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Grant No. AFOSR-85-0086

May 1986

Equipment Acquired:

The instrument purchased on the Air Force Office of Scientific Research grant 85-0086 was an Epics 741 Single Laser System Flow Cytometer from the Epics Division of the Coulter Corporation, Hialeah, Florida. The full purchase price of the instrument was $147,000. Pursuant to the provision entitled "cost sharing" of the AFOSR brochure the Grant amount was $126,635 and the Grantee (Ohio State University) cost sharing amount was $21,365.

Concise Summary of the Research Projects on Which the Instrument is Being Used:

The Epics 741 flow cytometer has and is being used in DOD projects entitled "Immune Dysfunctions and Abrogation of the Inflammatory Response by Environmental Chemicals" (AFOSR contract no. F49629-79-C-0163 and AFOSR grant no. #86-0129). The flow cytometry instrument is being used to evaluate the in vitro effects of unsymmetrical dimethyl hydrazine on the distribution and expression of T-lymphocyte subset antigens and Ia antigens.

The rationale for utilizing the Epics 741 instrument in these studies is based on the findings that different functional subsets of murine lymphocytes can be distinguished by their cell surface antigens. Specifically, murine T-lymphocytes can be divided into helper cells which have "Lyt. 1" antigens, and suppressor/cytotoxic cells which have "Lyt. 2, 3" antigens. Recently, highly specific monoclonal antibodies directed against these antigens have been developed and are now commercially available. These antibodies can be used to kill their target cells with complement or to visualize each subpopulation by use of fluorescein or enzyme-conjugated secondary reagents.

Alterations in the ration or absolute numbers of the human equivalent of Lyt. 1 and Lyt. 2, 3 (OKT4, OKT8, respectively) have been associated with certain autoimmune or immunodeficient diseases. The effects of UDMH on Lyt. antigens were previously evaluated using fluorescence microscopy, with equivocal results (one set of experiments suggested that UDMH enhances Lyt. 1 expression).

Now that the flow cytometer is available, these experiments are being rapidly repeated and will yield far more accurate results.

Research in the rapidly expanding field of immunogenetics has yielded several clues about the interaction of immunocytes responsible for an immune response. One important finding is the discovery of the immune response (Ir) gene which has been described in detail in the human and the mouse. This gene controls the expression of several cell-surface antigens including "Ia" antigens which are present in high density on B-cells, macrophages, and suppressor T-lymphocytes. Other T-lymphocytes (especially helper cells) have receptors for the Ia antigens. In order to mount the immune response, the antigen is generally "processed" and presented to T-cells and B-cells by macrophages. Recognition and interaction at the Ia site between the macrophage and lymphocyte (which must be genetically identical) is essential for an optimum immune response to occur. Similarly, for macrophages or suppressor T-lymphocytes to exert their regulatory (suppressive) action, there must be interaction between cells at the Ia site.

Since UDMH is an unstable molecule and has been shown to alter cellular membranes (Burns & Seiman), one mechanism for its immunomodulatory effects may be an interaction with or alteration of the Ia antigen and/or receptor site. We have previously examined the <u>in vitro</u> effects of UDMH on Ia antigen expression with the use of commercially available anti-Ia monoclonal antibody reagent and immunofluorescent microscopy. The results were again equivocal, but suggested that UDMH may cause enhancement of Ia antigen expression. These experiments are also being repeated using the flow cytometer, which should clarify the previous results.

It is anticipated that the acquisition of the Epic 741 flow cytometer will add an important new dimension to our understanding of the submolecular mechanisms of chemical-induced toxicity.

a) Calcium content of purified cell populations.

Calcium influx and intracellular calcium are currently being measured in cells from feline leukemia virus (FeLV)-infected cats and normal cat cells. The measurements are performed using the calcium-specific dye, Indo-1. Cells, when stimulated, allow calcium to flow across the surface membrane, when the Indo-1 binds free calcium there is a shift in its fluorescent absorption spectrum which is quantitated by the FACS. The cells currently under study include lymphocytes, neutrophils and macrophage. We have previously observed that these cells are inhibited by the presence of FeLV and we are determining if the observed inhibition is due to an interference in Ca^{2+} mobility.

b) Phagocytic cell degranulation and oxidative product formation.

Neutrophils degranulate rapidly upon stimulation by specific drugs. In addition, both neutrophils and macrophage produce oxidative products upon stimulation. We are presently measuring the loss of these functions in macrophage and neutrophils obtained from FeLV-infected cats and normal cells incubated with FeLV. Neutrophil degranulation is measured by staining the cells with acridine orange which stains the azurophilic granules of the cells red. Upon stimulation with the calcium ionophore A23187, the neutrophils degranulate and lose the red fluorescence. Other stimulators being used include latex beads, the peptide F-met-leu-phe and TPA. Oxidative product formation and phagocytes are being measured in neutrophils and macrophage from cats and mice using an enzyme reaction which, during an oxidative burst, changes the nonfluorescent 2'-7'dichlorofluorescein diacetate to fluorescent 2'-7'dichlorofluorescein. Texas red labeled Staph. A is used as a stimulator, which allows for quantitation of phagocytosis activity of the cells.

c) Quantitation of IL-2 receptor site on feline and murine lymphocytes.

We have previously shown that FeLV is able to block the activation of T-lymphocytes by interfering with interleukin 2 (IL-2) activity. Studies are

underway to determine the effect of FeLV on IL-2 receptor site numbers on feline and murine lymphocytes using fluoresceinated anti-IL-2 receptor antibody. In addition, we can simultaneously measure nucleic acid content of the cells using propidium iodide to determine any interference in cell cycling. The lymphocytes are stimulated with Concanavalin A and stained with the propodium iodide and anti-IL-2 antibody. The fluorescence can be measured simultaneously and numbers of cells in specific stages of the cell cycles and numbers of IL-2 receptor sites per cell can be quantitated.

d) Quantitation of natural killer (NK) cell activity in the presence of FeLV.

Natural killer cell activity can be quantitated using the FACS machine using carboxy-fluorescein diacetate-labeled target cells. The nonfluorescent diacetate compound is metabolized by functional cells to the free polar molecule which fluoresces. If the cell is lysed, the fluorescence is lost. In previous studies we have observed that human NK cells are inhibited by FeLV. We are currently studying the effect of FeLV upon NK cell lysis.

e) Quantitation and determination of cell type in the bone marrow of FeLV-infected cats.

Using available FITC-labeled anti-FeLV antibody, we are presently quantitating the level of expression of FeLV antigens in cats infected with FeLV. We are also quantitating the numbers and types of cells expressing the FeLV antigens compared to age and length of infection.

f) Use of Epics 5 for non-FeLV related studies.

1) Effect of 8-methoxypsoralen on human interleukin 2 receptor formation.

These studies involve the use of normal human lymphocytes stimulated with mitogen in the presence or absence of 8-methoxypsoralen (8-MOP). Previous studies have shown a loss in lymphocyte stimulation in the presence of 8-MOP which was associated with a loss of IL-2 activity. In order to determine if the

IL-2 associated effect was due to a loss of IL-2 receptors, a study was performed using FITC-labeled anti-IL-2 receptor antibody to quantitate the numbers of receptor sites per cell. We found that 8-MOP was able to decrease the total number of IL-2 receptor sites per lymphocyte in a dose-dependent fashion.

g) Isolation of subsets of cells expressing Ia antigen by the cell sorting technique.

In this study, rare subsets of LCL cells which express Ia antigens are being separated and purified using the flow cytometric cell sorting capacity. Live LCL cells are stained for membrane antigen by indirect fluorescent antibody technique using Ia-specific antiserum. In the cell population, approximately 1 in 100 will express antigen. The instrument is adjusted to sort cells based on the magnitude of fluorescence. Ia-positive (fluorescing) cells are then separated from the general cell population by the cell sorting device. These cells are collected in tissue culture media under sterile conditions and later propagated. By this method these rare Ia-positive cells can be expanded in tissue culture and subsequently cloned.

h) Retrovirus-associated cell antigen expression during various stages of cell cycle.

Cell cycle/antigen expression studies have been performed on cell lines productively infected with feline leukemia virus (FeLV) or human T-cell lymphotropic virus (HTLV-I). In each case membrane antigen expression was detected by indirect immunofluorescence using convalescent antisera primary while cell cycle was determined by propidium iodide (P.I.)-stained DNA.

For these studies, live cells were first stained for antigen using FITC-conjugate reagent. The cells were fixed then stained with P.I. Finally, the cell preparations were analyzed by flow cytometry.

From these experiments we have determined that retroviral virus antigen expression occurs throughout the cell cycle but reaches its peak during the G_2/M

phase of the cell cycle. The results from this study define the best time to harvest cells for optimum membrane antigen yield.

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