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Review

Flow Cytometric Evaluation of Leukocyte Function

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The marriage of monoclonal antibody technology and flow cytometry has provided clinical researchers with a powerful tool for the characterization of leukocytes from various sources. In this regard, flow cytometry has been primarily used for the immunophenotyping of peripheral blood lymphocytes in leukemias and various immunodeficiency disorders. Flow cytometry is also useful for the evaluation of leukocyte function in vitro and in vivo. This review discusses the various applications of flow cytometry for the assessment of leukocyte function. Since several cell surface antigens are important constituents involved in cell function, immunofluorescence identification of these markers can provide significant information regarding cell function. Analysis of activation antigen expression by monoclonal antibodies and flow cytometry can provide significant insights about the presence of functional leukocyte populations in patients. Flow cytometry can also be used to directly analyze leukocyte function. Procedures for the quantitative flow cytometric analysis of proliferation of activated lymphocyte subsets are reviewed. Early cell activation is also amenable to flow cytometric measurement. Early activation events such as alterations in membrane potential, intracellular free calcium redistribution, intracellular pH, and changes in membrane fluidity, as well as the direct measurement of enzyme activity, are also described.

Key words: flow cytometry, leukocyte function, immunofluorescence, membrane fluidity, membrane potential

INTRODUCTION

Without a doubt, the most frequent application of flow cytometry has been the quantitation of cell surface markers by immunofluorescence. This has clearly been the case concerning clinical applications of cytometry, where characterization of cell phenotype with monoclonal antibodies and flow cytometry has become a routine procedure for the evaluation of hematopoietic neoplasms in many diagnostic pathology laboratories [1].

While flow cytometers are valuable tools for the sorting and purification of cells for further functional testing by other methodology, the purpose of this review is to explain the varied uses of flow cytometry for the evaluation of cell function. It is an overview of the various possibilities for leukocyte function testing by flow cytometry, along with potential clinical applications.

For this discussion, the working definition of cell function is as any physiologic activity performed by cells that requires active cellular metabolism. The various functional interactions that take place between cells, especially cells of the immune system, all require metabolic activity in at least some capacity. Traditionally, cell function studies are performed on entire populations of cells by tedious manual methods. A cell suspension will demonstrate functional activity if enough cells in the test tube are capable of performing the function in question.

The most valuable aspect of flow cytometry is the ability to make measurements on individual cells. Because of this unique capability, the flow cytometric assessment of cell function allows one to identify heterogeneous populations of cells with varying degrees of functional activity rather than simply determining the average functional level of a group of heterogeneous cells. Depending upon the parameter measured, information regarding cell function can be obtained either by indirect observation of the effects or by-products of cell functions, or by the direct quantitation of actual physiologic activity. Some of the

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currently available assays of cell function have clear implications in the clinical management of patients, while others described in this review are still at an early stage of investigation with respect to clinical utility.

ANALYSIS OF LEUKOCYTE FUNCTION BY IMMUNOFLUORESCENCE

It has long been the goal of immunologists to be able to discriminate functional subpopulations of lymphocytes based upon phenotypic characterization of membrane antigens. The early work describing the division of functional murine helper and suppressor T cells based upon the expression of the LyT-1 and LyT-2 antigens [2] stimulated considerable research and development in an effort to produce reagents that effectively define functional lymphocyte subpopulations. In this regard, cell surface phenotyping of human lymphocyte subpopulations has not proven as definitive in the assessment of immunocompetence as originally hoped [3]. Simply enumerating the types of cells with functional potential is not the same as actually defining their ability to perform that function. However, immunofluorescence phenotyping remains a useful tool for the assessment of cell function since the quantitation of structural parameters such as membrane antigen expression can provide information directly related to the functional activity of a cell population of interest.

Since cell surface proteins are integral components in the various physiologic responses of the cell, activation of a cell to perform any given function frequently results in the alteration of the matrix of glycoprotein antigens displayed on the cell membrane. These alterations may take the form of the appearance of new protein structures, or in the increase or decrease in antigens normally displayed by the cell in the resting state. Thus, the immunofluorescent characterization of carefully selected antigens related to cell function can provide useful information about the activation state and functional capability of cells.

A prime example of neoantigen expression upon cell activation is the cell surface receptor for interleukin 2. T cell responses to antigen or mitogen depend upon both the synthesis of the regulatory hormone interleukin-2 (IL2) as well as on the expression of a receptor for the molecule [4–6]. Recent evidence also indicates that the expression of this membrane receptor may be important in the proliferation and differentiation of certain types of B cells [7,8] as well as in the activation of monocytes and macrophages [9–11].

Multicolor immunofluorescence and flow cytometry is ideally suited for the characterization of activation markers such as IL2 receptors on various subpopulations of cells. Thus, because the activation and subsequent response of T cells is dependent upon the obligate expression of IL2 receptors on functionally competent cells, the phenotypic demonstration of interleukin-2 receptors on T cells, by definition, identifies an activated population of cells. The quantitation of IL2 receptor expression by flow cytometry has in fact been shown to be a rapid and sensitive method for the analysis of the in vitro response of T cells to mitogen and antigen and has been used for the evaluation of immunocompetence in patients with autoimmune deficiency syndrome and related immunodeficiency disorders [12].

Immunofluorescence flow cytometry is useful in characterizing a number of other cell surface markers expressed de novo by functionally active T cell subsets. Activation antigens such as 4F2 [13], the transferrin receptor [14], and the expression of class II histocompatibility antigens on T cell subsets [15,16] are useful in the characterization of functionally active T lymphocyte subpopulations, both in vitro [15–18] and in vivo [16,19–22].

Monoclonal antibodies are also available that define antigens on B cells activated by antigen or mitogen [7,8,23]. Immunofluorescent characterization of B cells demonstrating increased expression of these activation markers may lend some insights into the role of B cell activation in certain autoimmune diseases and other clinical conditions in which humoral responses play a role in pathology.

The quantitation of constitutively expressed antigens can also provide information regarding the activation level or function of various cell types. Flow cytometry is particularly well-suited for this type of investigation because changes in antigen density are reflected as variations in fluorescence intensity when quantitated on the flow cytometer.

A good example of this specific application of immunofluorescence in leukocyte function testing is the correlation of neutrophil function with the quantitation of the cellular expression of the type III complement receptor (CR3, CD11). The CR3 receptor belongs to a family of glycoproteins associated with cell adhesion and specifically mediates the adherence and phagocytosis of particles opsonized with iC3b [24–27]. The activation of neutrophils with various stimuli such as phorbol esters [28] and opsonized bacteria induces the increased cell surface display of this constitutive membrane receptor.

The demonstration of altered neutrophil CR3 expression by immunofluorescence flow cytometry correlates with the functional response of the cell to biologic stimuli. For example, the activation of complement by new hemodialysis membranes leads to increased CR3 expression and neutrophil activation as a result of the generation of biologically active complement split products [29]. A genetic abnormality in the synthesis and expression of CD11 is integral in the etiology of a recently described

clinical syndrome [28,30]. Patients with this neutrophil defect suffer from chronic recurrent infections related to an inability to phagocytize C3 opsonized particles.

Activation of monocytes and macrophages by various ligands also results in the modulation of several different cell surface markers. Class II histocompatibility antigens [31], CD11 antigens [32,33], and receptors for the Fc region of immunoglobulin G [34] are examples of antigens expressed with increased density on activated cells of this lineage.

DIRECT MEASUREMENT OF CELL FUNCTION BY FLOW CYTOMETRY

Cell Kinetics

While it is apparent that the quantitation of appropriate antigens by immunofluorescence can yield important information pertaining to cell function, flow cytometry is also a powerful tool for the direct assessment of physiologic activity in leukocytes. Many fluorescent probes are available that directly bind to molecules integral to physiologic responses of leukocytes and therefore are amenable to use in the flow cytometric quantitation of various cell functions.

One of the most basic cell functions amenable to flow cytometric quantitation is the proliferative response of lymphocytes to antigenic or mitogenic stimulation. Traditionally, lymphocyte transformation assays are performed employing the incorporation of radioactive nucleotides into newly synthesized DNA as a way to quantitate the degree of proliferation in response to various agents.

A number of flow cytometric methods are available for the quantitation of cell cycle phase based on the staining of cellular DNA content with various DNA specific fluorochromes (for review, see [35]). The basic approach involves the staining of lymphocyte DNA, or DNA and RNA in combination, so that the degree of dye fluorescence is directly proportional to the amount of DNA present in the cell. There are many different DNA stains available for the quantitation of lymphocyte proliferation, but the basic methodology is similar in all cases. Cells are stimulated with antigen or mitogen and cultured for varying periods of time after which they are removed from culture and stained with the DNA dye of choice. The cells are then analyzed on the flow cytometer, and the number of cells in proliferative phases of the cell cycle are determined. Normal unstimulated lymphocytes have a diploid amount of DNA and produce a fluorescence histogram with a small coefficient of variation. Responding lymphocytes begin to synthesize new DNA and thus stain proportionally brighter as they traverse the cell cycle toward G_2/M. At the point just before mitosis, there is exactly twice as much DNA as that found in the resting unstimulated lymphocyte.

In addition to characterizing in vitro lymphoproliferative responses to mitogen or antigen, the determination of cell cycle phase by flow cytometric DNA content analysis can be useful in identifying actively proliferating lymphocytes in various tissues in an attempt to correlate lymphocyte activation and proliferation with pathology. Noteworthy are studies that have demonstrated that patients with active multiple sclerosis have actively proliferating lymphocyte populations in spinal fluid [36].

Actively proliferating cells can also be identified by immunofluorescence procedures. These techniques more directly parallel the traditional DNA radiolabeling procedures. In one application, proliferating cells, cultured in the presence of bromodeoxyuridine (BRDU), will incorporate the BRDU into newly synthesized DNA. By the use of a monoclonal antibody reactive with the segments of chromatin that have incorporated the BRDU, nucleotide-labeled cells are easily identified by immunofluorescence. Simultaneous analysis of cells for total DNA content and BRDU immunofluorescence clearly identifies the cells in S phase and even allows for the determination of doubling times of cultured cells [37–40]. Stevenson et al. [4] used the BRDU assay as the basis for a technique to measure the cytostatic potential of activated macrophages against cultured tumor cells.

Monoclonal antibodies are also available that react with nuclear proteins expressed only during the proliferative phase of the cell cycle [42,43]. Utilization of these antibodies for the analysis of proliferating cells is analogous to the technique of anti-BRDU immunofluorescence with the exception that it is unnecessary to pulse the cells with a nucleic acid analog before analysis.

Because flow cytometry offers the ability to simultaneously measure multiple parameters on individual cells, it is possible to combine techniques of cell surface phenotyping with the concomitant analysis of cell cycle phase. This methodology allows the investigator to determine the proliferative state of individual leukocyte subsets as recognized by selected monoclonal antibodies to cell surface differentiation antigens. In a true multiparameter approach to the analysis of proliferating cells, Houck and Loken [44] combined DNA content analysis, cell surface phenotyping, and BRDU incorporation to analyze the proliferating subpopulations of cells in the mouse thymus.

To define better the phenotype of functional T cell subsets, Cottner et al. [45] used a combination of Hoechst 33342 DNA staining and fluoresceinated monoclonal antibodies to identify antigens expressed on subpopulations of T cells responding to lectin stimulation as well as on alloreactive T cells [17]. Using this approach for the identification of proliferating T cell subsets, Williams et al. [21] quantitated activated lymphocyte subsets in patients undergoing acute renal allograft rejection, thereby demonstrating the potential clinical relevance of this application of multiparameter flow cytometry for the iden-

tification of functionally important cells. Recently, one of the authors (G.T.S.) has used a similar technique to identify the phenotype of proliferating lymphokine-activated killer cells (LAK cells) in bulk cultures of lymphocytes treated in vitro with high dose IL-2 before administration of the cells to cancer patients [46].

Thus, flow cytometric assessment of cell proliferation subsequent to the stimulation of cells by various agents offers not only the capability of determining the precise cell cycle distribution of responding cells, but also the ability to determine cell cycle time with BRDU analysis, as well as the phenotype of the responding cells. This is certainly an improvement over the determination of the average DNA synthesis of a heterogeneous cell population.

Flow Cytometric Analysis of Neutrophil and Monocyte Function

In addition to the evaluation of lymphocyte function both in vitro and in vivo, flow cytometry is valuable for the direct measurement of certain aspects of neutrophil and monocyte function. In fact, one of the first cell function assays adapted for flow cytometry was the analysis of phagocytic function by these cells. Numerous modifications of techniques for the flow cytometric evaluation of phagocytosis have been described. However, they all basically rely on the quantitation of the number of fluorescent particles ingested by various phagocytic cell types [47–49]. Fluorescent particles used have included latex microbeads, dye-labeled bacteria, and yeast particles. When the coefficient of variation of the target particle is sufficiently small, the numbers of particles ingested can be determined based upon the fluorescence intensity of the ingested particles [50]. It is also possible to discriminate between intracellular particles and particles attached to the cell but not actually internalized by the use of a fluorescence quenching technique described by Bjerknes [48]. In this procedure, trypan blue dye added to the suspension of cells and fluorescein-labeled bacteria before analysis on the flow cytometer quenches the fluorescein fluorescence of all extracellular bacteria but has no effect on the fluorescence of internalized organisms [48].

The stimulation of phagocytic cells by certain bacterial products induces the resting cell to undergo a metabolic change that results in an increased oxidative metabolism and the generation of toxic by-products of oxygen. This oxidative respiratory burst is in large part responsible for the killing of ingested bacteria as well as a major portion of tissue damage that results from acute inflammation. Bass et al. [51] have recently described a flow cytometric assay for the quantitation of neutrophil oxidative respiratory burst. In this assay, isolated neutrophil or buffy coat leukocytes are incubated with 2,7-dichlorofluorescin diacetate (DCF-DA), a nonfluorescent analog of fluorescein diacetate. Owing to its nonpolar nature, the DCF-DA readily traverses the cell membrane. Cytoplasmic esterases act to desacetylate the DCF-DA to form the polar molecule 2,7-dichlorofluorescin, which becomes trapped intracellularly. The generation of hydrogen peroxide during the respiratory burst results in the conversion of the nonfluorescent dichlorofluorescin to highly fluorescent dichlorofluorescein (DCF). The flow cytometer measures the extent of the respiratory burst by quantitating the green DCF fluorescence generated in stimulated DCF-DA-loaded cells compared with unstimulated DCF-DA-loaded cells. The assay is in many ways analogous to the nitroblue tetrazolium dye reduction test used in the screening of patients for leukocyte oxidase deficiency, such as that found in chronic granulomatous disease.

More recently published modifications of the DCF assay make optimal use of the multiparameter capabilities of flow cytometry to simultaneously measure oxidative metabolism and phagocytosis [52,53]. Incubation of Texas red dye-labeled *Staphylococcus aureus* with DCF-DA-loaded neutrophils allows the quantitation of the metabolic response of large numbers of individual cells to a phagocytic challenge. Phagocytosis is measured by the generation of red fluorescence owing to ingested organisms, which in turn trigger the oxidative burst and subsequent development of green DCF fluorescence.

The assays of neutrophil function described above have the most potential for routine clinical application. Analogous assays, such as the nitroblue tetrazolium reduction assay, have been in clinical use for a number of years for the screening of neutrophil defects in patients with chronic recurrent infections. The development and application of similar flow cytometric procedures for this purpose should allow for the quantitative analysis of functional defects in cells from animals or patients with abnormal responses to infection [54–57]. This should prove particularly useful when coupled with assays for the detection of cell surface products such as the CD11 antigen, which are integral in the function of phagocytic cells.

FLOW CYTOMETRIC ANALYSIS OF EARLY CELL ACTIVATION

Membrane Potential

In its most basic sense, the essence of cell function testing is the measurement of the cell's physiologic response to various external stimuli. Regardless of the specific effector function of interest, there are certain basic metabolic alterations that must occur in cells of all types in order for the cell to exert its final functional activity. For example, cellular responses such as DNA synthesis or the generation of toxic oxygen by-products and phagocytosis are really only the end-stage component of a com-

plex cascade of intracellular metabolic events that must transpire in order for the cell to achieve its ultimate functional potential.

One of the more exciting new applications of flow cytometry is the measurement of very early events in leukocyte activation. Since most of the cell functions of clinical interest develop following an initial encounter between an extracellular stimulant and the cell membrane, one of the earliest indications of cell responsiveness is an alteration in the membrane potential of the responding cell. Shapiro et al. [58] recently described a technique for determining changes in lymphocyte membrane potential in a flow system using fluorescent carbocyanine dyes.

Resting cells are effectively electronegative with respect to the extracellular environment as a result of the normal electrochemical gradient of Na^+, K^+, and Cl^- ions across the cell membrane. The carbocyanine dyes, such as dihexyloxacarbocyanine [$DiOC_6(3)$], are highly lipophilic and cationic at physiologic pH. $DiOC_6(3)$ freely passes the hydrophobic cell membrane and becomes partitioned between the cytoplasm and the exterior of the cell based upon the concentration gradient of the dye as determined by the membrane potential. Once the cell has reached equilibrium with the probe, interaction of the cell with ligands that cause depolarization results in a decreased electronegative charge in the cell and a reflux of the cationic dye from the intracellular to the extracellular environment with a resulting decrease in intracellular fluorescence. Conversely, hyperpolarization of the cell results in an increased net negative charge on the cell and a redistribution of the dye from the extracellular space across the membrane and into the cytoplasm, resulting in a net increase in intracellular fluorescence.

Since alterations in membrane potential occur in seconds to hours following interaction of sensitive cells with a ligand, flow cytometric analysis of membrane potential changes may have applications in the rapid assessment of cellular responses that require days to perform with standard methodology. Lymphocyte responses to soluble antigens or mitogens [35] or the early detection of mixed lymphocyte responsiveness to class II histocompatibility antigens for the evaluation and selection of potential donors of cadaveric organs for transplantation are two examples of applications in which the quantitation of membrane potential changes may allow for the early detection of cell responsiveness. It may also be feasible to use alterations in membrane potential as a rapid screen for the effect of new pharmacologic agents on cellular growth parameters such as clonogenicity [59]. Recent studies by Seeds et al. [60] demonstrated the utility of membrane potential measurements in the analysis of defects in neutrophil function. These investigators combined flow cytometric procedures for the oxidative respiratory burst with the measurement of membrane potential to study the relationship between alterations in membrane potential and the initiation of oxidative metabolism in stimulated neutrophils.

Intracellular Free Calcium Redistribution

One of the more commonly shared physiologic events in the activation pathways of many different cell types is the breakdown of membrane phospholipids subsequent to ligand binding. This phospholipid degradation results in the mobilization of calcium from intracellular stores, with a rapid rise in cytoplasmic free Ca^{+2}. The regulation of intracellular free Ca^{+2} levels plays a central role in the cascade of diverse metabolic pathways that lead to the eventual expression of cell function. The recent development of fluorescent dyes specific for free Ca^{+2}, particularly a new dye called Indo-1, has made possible flow cytometric analysis of calcium redistribution associated with the early phase of leukocyte activation.

Single cell analysis of intracellular calcium redistribution is a powerful technique for the demonstration of T cell [61–63] and B cell [64,65] activation subsequent to cross-linking of lymphocyte antigen receptors with divalent monoclonal antibodies. Indeed, in an elegant study demonstrating the full potential of multiparameter flow cytometry, Rabinovitch et al. [66] coupled the use of Indo-1 staining with immunofluorescence to demonstrate differences in the onset, duration, and magnitude of the Ca^{+2} flux in specific T cell subsets in Indo-1 loaded cells concomitantly stained with phycoerythrin-labeled subset-specific monoclonal antibodies.

Membrane Fluidity

Studies on the relationship between stimulus-response coupling and cell membrane structure may provide important information relating to cell function. For some years the concept of the cell membrane as a fluid mosaic [67] provided a basis for studies involving receptor binding and signal transduction across the membrane.

The use of membrane fluidity or microviscosity as a measure of cell status or function is becoming more prevalent with the availability of good fluorescent dyes such as diphenyl hexatriene (DPH) and its analog trimethylammonium diphenylhexatriene (TMA-DPH). Diphenylhexatriene is a polyene hydrocarbon that is uncharged and hydrophobic and therefore partitions into lipid, primarily in the plasma membrane [68] but also into most lipid-containing structures [69,70]. When excited by ultraviolet (UV) light, DPH emits fluorescence measurable at 430 nm. Webber [71] and Shinitzky [72,73] previously described in detail the theory for determination of membrane fluidity in biological membranes. There are a number of reports of the use of flow cytometry and the DPH probe for fluidity determinations [74–76]. TMA-DPH appears to be ideal for flow cytometric evaluation

of microviscosity since it loads rapidly (3 min) primarily into the outer membrane of cells.

A number of studies have demonstrated that there is an important relationship between the microviscosity of neutrophil membranes and the affinity and number of some membrane receptors such as those for formyl peptides [77,78]. Tomonaga et al. [78] clearly demonstrated that formyl peptide receptor expression increased as neutrophil membranes were fluidized with aliphatic alcohols or cis-vaccenic acid. Work in our laboratory (J.P.R.) indicates that human peripheral blood monocytes demonstrate an increased fluidity during culture concomitant with increasing phagocytic ability. Additionally, events such as superoxide production are strongly related to the physical restructuring of the cell membrane upon activation. This suggests that production of neutrophil oxidative products may be closely related or dependent upon a conformational alteration in the membrane structure.

Membrane structure also plays an important role in lymphocyte activation. A recent study of spleen B cells demonstrated that endocytosis of membrane IgG is inhibited by increasing rigidity but not increasing fluidity [79]. While this study was primarily concerned with the effect of addition of rigidifying or fluidizing agents per se, the study provides support for the concept that cellular activation is related to the membrane structural status. Measurements of membrane fluidity may, therefore, be predictive of cellular activation status. A clear relationship has been established between phagocytosis and the organization of membrane lipids by demonstrating a fluidizing change during phagocytosis [80,81]. Further information about the heterogeneity of functionally active cells should be obtained from experiments using the multiparameter capabilities of flow cytometry to couple measurements of cell membrane fluidity with the phenotypic identification of cell subpopulations as defined by fluorochrome-labeled monoclonal antibodies.

Intracellular pH

There has been a steady increase in the use of flow cytometry in the analysis of activation-related changes in intracellular pH since the initial report of the application of the technology for the measurement of pH in single cells [82]. Alterations in cytoplasmic pH frequently accompany early changes in cellular physiology subsequent to the response of the cell to an activation signal. Several dyes have been shown to be sensitive indicators of intracellular pH either by pH-dependent shifts in excitation or emission levels. Vissers et al. [82] observed that the alteration in excitation spectra of fluorescein (diacetate) was dependent upon changes in pH. Valet et al. [83] used the ultraviolet dye 1,4-diacetoxy-2,3-dicyanobenzene for the determination of intracellular pH. The ratio of the green and blue emission of the ultraviolet excited dye is pH dependent. Other fluorochromes such as carboxyfluorescein and 4-methylumbelliferone have also been used for the estimation of cytoplasmic pH by flow cytometry [35, 84]. Recently, a new probe 2′,7′-bis-carboxyethyl-5(6)-carboxyfluorescein (BCECF) has been described, which has pH-related dual emission spectra upon excitation at 488 nm [63]. This probe will make the measurement of intracellular pH by flow cytometry a technique more readily adaptable by more laboratories than those methods requiring dual laser excitation.

A good example of the application of this methodology to neutrophil function testing is the report by Bassoe et al. [85]. These experiments demonstrated an elegant method for simultaneously measuring phagocytosis and pH since the fluorescence of FITC-labeled *Staphylococcus aureus* was shown to be pH dependent. Once phagocytosed, the fluorescence intensity of ingested FITC-labeled organisms changed as the microbes became localized in phagolysosomes. The changes in *S. aureus* fluorescence correlated with intraphagosomal pH.

The activation of resting T lymphocytes also results in alterations in intracellular pH. In experiments reported by Alcover et al. [63], BCECF-loaded helper T cell clones demonstrated early changes in intracellular pH when stimulated with antibodies to CD3 but not with antibodies to CD4 or CD8. The demonstrated rise in cytoplasmic pH occurred very early in the activation process and was closely related to the redistribution of intracellular calcium in ligand-stimulated cells. Thus, like the measurement of intracellular calcium redistribution described earlier, the flow cytometric analysis of intracellular pH provides information on the very early stages of cellular activation.

Measurement of Cellular Enzyme Activity

Since intracellular enzymes play a crucial role in nearly all stages of cellular physiology, the quantitation of function-related enzyme activity should impart significant information regarding cell activation and function. However, the measurement of cytochemical events within single cells by flow cytometry has not progressed significantly despite the potential value in this concept. The earliest measurements of enzyme activity by flow cytometry are by Dolbeare et al. [86–88]. One such technique measured the presence of phosphatases and glucuronidases using naphthol derivatives as fluorogenic substrates. Blair et al. [89,90] demonstrated the presence of oxidative burst enzymes in HL-60 cells in a series of experiments in which they simultaneously measured the reduction of nitroblue tetrazolium dye, cell cycle phase, and phagocytosis.

Several other publications attest to the efficacy of flow cytometry for the measurement of cellular enzyme activity [91,92]. Esterase activity is easily measured using DCF-DA [51,56] or carboxyfluorescein diacetate. The latter is directly hydrolysed to green fluorescent carboxy-

fluorescein by cellular esterases [93]. Such fluorochromes are useful indicators of the presence of esterases in many cell types.

CONCLUSIONS

Currently, the predominant clinical application of flow cytometry is the quantitation of lymphocyte surface markers by monoclonal antibodies. This application has unquestionable value in the characterization and classification of hematologic malignancies as well as for the evaluation of patients with suspected immunologic disorders. In addition, this application has enabled the technology of flow cytometry to become established as a productive part of the diagnostic pathology laboratory.

The use of flow cytometry for the evaluation of cell function is a promising new area with great potential for application in the clinical laboratory setting. The ability of the flow cytometer to make multiple measurements on single cells has allowed clinical investigators to begin to examine the functional heterogeneity of cells in much the same way that monoclonal antibodies and flow cytometry have advanced our understanding of the diverse pathways of leukocyte ontogeny and differentiation.

Hopefully, this discussion has acquainted the reader with some of the various possibilities for cell function testing by flow cytometry. It is also hoped that an increased awareness of this untapped potential for the technology will stimulate further investigation into the clinical applications of cell function testing by flow cytometric analysis. While some of the techniques described here are currently beyond the scope of many clinical cytometry laboratories, the future holds the promise of rapid measurement of the most fundamental aspects of the physiology of cell activation in the evaluation of lymphocyte responsiveness, phagocytic cell function, and the response of cells to various pharmacologic agents. Only further research and development will determine whether this technology has a place in the routine clinical analysis of leukocyte function.

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