The detection and quantification of apoptotic cells is becoming increasingly important in the investigation of the role of apoptosis in cellular proliferation and differentiation. The pathogenesis of hematologic disorders such as aplastic anemia and the development of neoplasia are believed to involve dysregulation of apoptosis. To quantitate accurately the proportion of apoptotic cells within different cell types of a heterogeneous cell population such as blood or bone marrow, a method is required that combines the analysis of large numbers of cells with concurrent immunophenotyping of cell surface antigens. In this study, we have evaluated such a method using the fluorescent DNA binding agent, 7-amino actinomycin D (7AAD), to stain three diverse human cell lines, induced to undergo apoptosis by three different stimuli. Flow cytometric analysis defines three populations on the basis of 7AAD fluorescence and forward light scatter. We have shown by cell sorting and subsequent morphological assessment and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling that the populations defined by 7AAD represent live, apoptotic, and late-apoptotic/dead cells. This method is quick, simple, reproducible, and cheap and will be a valuable tool in the investigation of the role of apoptosis in normal physiology and in disease states.

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A POPTOSIS, OR PROGRAMMED cell death, is a critical process in the regulation of cellular proliferation and differentiation. In normal hemopoiesis, for example, apoptosis is involved in regulating the rate of committed cell production through the sensitivity of progenitor cells to the presence or absence of survival factors. Withdrawal of normal survival factors leads to apoptosis of progenitor cells.

The pathways of cellular proliferation and apoptosis appear to be inexorably linked to minimize the occurrence of neoplasia. Dysfunction of apoptosis has been proposed as a pathogenetic process in bone marrow disorders such as aplastic anemia, malignant tumors, and the pathology of viral infections (such as human immunodeficiency virus [HIV]).

Examining the role of apoptosis in both health and disease states requires a method for the detection and quantitation of apoptotic cells within a heterogeneous population, which should be sensitive, specific, and simple to perform on multiple samples. Staining with 7-amino-actinomycin D (7AAD) fulfills these criteria and allows dual staining for cell surface antigens, so that apoptosis within subpopulations can be assessed.

Apoptosis is characterised by certain morphological features (cell and nuclear shrinkage, cytoplasmic blebbing, and nuclear and cytoplasmic fragmentation) and, in most cases, by DNA fragmentation producing the classic DNA ladder on agarose gel electrophoresis. This is caused by the activation of a specific endonuclease, which cleaves DNA into nucleosome-sized fragments of 180 bp or multiples thereof. There are many ways of detecting apoptosis, but all have disadvantages, particularly in quantitating numbers of apoptotic cells. Methods include (1) simple morphological assessment to detect features outlined above using light or electron microscopy or time-lapse photography and (2) the detection of DNA fragmentation by gel electrophoresis or by terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL).

The use of flow cytometry-based methods of detecting apoptotic cells has the advantage that large numbers of cells can be rapidly and accurately examined and allows for easy quantitation of specific populations. Most methods involve permeabilization of the cell membrane and the use of fluorescent DNA-binding agents to detect the lower DNA content of the apoptotic cell nucleus, eg, staining with propidium iodide (PI).

7AAD is a fluorescent DNA-binding agent, which intercalates between cytosine and guanine bases. In combination with Hoechst 33342, it has been used to define dead (7AAD-bright), apoptotic (7AAD-dim), and live (7AAD-negative) populations by flow cytometry; however, this requires cell fixation and UV-laser excitation. Schmid et al have shown that, used alone to stain unfixed cells, 7AAD can define the three populations without the need for dual staining with Hoechst 33342. Discrimination of the three populations was validated by cell sorting and morphological examination alone.

In this study, 7AAD was used as a single agent to detect apoptotic cells by fluorescence-activated cell sorting (FACS) so that large numbers of cells could be analyzed simply and quickly. To validate the method described by Schmid et al, three human cell lines were studied, (1) a T-cell leukemia cell line known to undergo apoptosis rapidly after treatment with topoisomerase inhibitors; (2) a growth factor-dependent hemopoietic progenitor cell line that undergoes apoptosis on growth factor withdrawal, perhaps more analogous to the occurrence of apoptosis in BM; and (3) an adherent, drug-resistant carcinoma cell line, which requires prolonged drug treatment to induce apoptosis. Live, apoptotic, and dead populations were defined on the basis of 7AAD staining, and these results were validated by cell sorting, followed by

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analysis with a variety of established methods for detecting apoptotic cells, including DNA gel electrophoresis, TUNEL, morphological assessment, and time-lapse photography.

MATERIALS AND METHODS

Cell culture. The human T-cell leukemia cell line JURKAT was cultured in RPMI-1640 (Sigma, Poole, UK), supplemented with 1% penicillin/streptomycin, 1% glutamine, and 10% fetal calf serum (FCS; Life Technologies, Paisley, UK). The human growth factor-dependent erythroleukemia cell line TF-1 was grown in RPMI-1640, supplemented as above and with 2 to 5 mg/mL granulocyte-macrophage colony-stimulating factor (GM-CSF; 1.4 X 10⁵ U/mg; Sandoz Pharma, Basel, Switzerland). The human pancreatic carcinoma cell line T3M-4 was cultured in RPMI-1640, supplemented with 10% FCS, 1% glutamine, and 50 μg/mL gentamicin sulphate (Sigma). This adherent cell line was maintained in a monolayer and subcultured every 2 to 3 days using trypsin-EDTA (ethylenediaminetetraacetic acid disodium salt; Sigma).

Induction of apoptosis. JURKAT cells were treated with various concentrations (1 to 30 μg/mL/10⁶ cells) of camptothecin (Sigma), a topoisomerase-I inhibitor, for 4 hours at a cell concentration of 0.5 X 10⁶/mL. TF-1 cells were cultured in the absence of GM-CSF, at 0.5 X 10⁶ cells/mL for up to 4 days. Subconfluent T3M-4 cells were treated with 10 μg/mL and 30 μg/mL of camptothecin for 6 to 48 hours at 37°C. After treatment, the nonadherent T3M-4 cells were removed, and the monolayer was trypsinized. The trypsinized cells were combined with the nonadherent cells before staining.

Cells were then washed twice in phosphate-buffered saline (PBS), containing 0.05% azide and 1% FCS, and were resuspended in PBS at a concentration of 1 X 10⁶/mL, ready for staining. Cell viability was assessed by trypan blue (Flow Labs, Irvine, UK) staining.

7AAD staining and flow cytometry. 7AAD (Calbiochem-Novabiochem, Nottingham, UK) was dissolved in acetone and diluted in PBS to a concentration of 200 pg/mL. This was kept at -20°C and protected from light until use. A total of 100 μL of 7AAD solution was added to 10⁶ cells suspended in 1 mL PBS and was mixed well. The cells were stained for 20 minutes at 4°C while protected from light and then were pelleted by centrifugation. The supernatant was removed, and the cells were resuspended in 500 μL 2% paraformaldehyde (PF) solution (Sigma). Unstained fixed cells were used as negative controls. Samples were analyzed on a FACStar cell sorter (Becton Dickinson, Mountain View, CA) within 30 minutes of fixation. The whole nucleated cell population was analyzed. Data on 50,000 cells was acquired and processed using Lysys II software (Becton Dickinson). Scattergrams were generated by combining forward light scatter with 7AAD fluorescence, and regions were drawn around clear-cut populations having negative, dim, and bright fluorescence. Cells within these regions were sorted, first in Enrich mode and subsequently in Normal-R mode. The frequency of cells with low, medium, and high 7AAD fluorescence was assessed both pre-FACS and post-FACS sorting and the purity and enrichment of the sorted populations were then calculated. The sorted populations were then used in other assays of apoptosis described below.

Morphological assessment. Cytospin preparations (10⁶ cells) of unsorted and sorted populations were stained with May-Grunwald-Giemsma (MGG) stain.

TUNEL. Cytospin preparations (10⁶ cells) were fixed for 15 minutes in fresh 4% PF solution. They were then washed and stored in 80% ice-cold ethanol at -20°C until staining (up to 6 weeks). Slides were then washed well in PBS, and 50 μL reaction mix (10% 2.5 mmol/L cobalt chloride, 20% buffer [0.2 mmol/L potassium cacodylate, 25 mmol/L Tris, and 0.25 mg/mL bovine serum albumin], 2% TdT (TdT Kit; Boehringer Mannheim, Lewes, UK), 2% fluorescein isothiocyanate [FITC]-conjugated dUTP [Fluorogreen; Amersham, Little Chalfont, UK] and 66% distilled water) was loaded onto each slide.

Negative controls consisted of slides incubated in reaction mix lacking TdT. Slides were incubated for 60 minutes at 37°C while protected from light. After this time, they were washed well in PBS, mounted in a solution of 48% PBS, 48% glycerol, and 4% formaldehyde, and examined using the fluorescence microscope (wavelength, 488 nm). The percentage of apoptotic cells was recorded and a visual record made photographically. Some samples were examined by in situ nick translation using the ApoTag peroxidase-detect dase kit (Onscor, Eastleigh, UK), according to the manufacturer’s instructions.

DNA gel electrophoresis. DNA was extracted from 10 X 10⁶ cells by the Tri-Reagent method (Molecular Research Center, Cincinnati, OH). Briefly, cells were lysed in 1 mL Tri-Reagent. Chloroform (0.2 mL) was added, and the sample was centrifuged at 14,000 rpm at 4°C for 15 minutes. The aqueous phase was separated (RNA was subsequently extracted from this phase), and 0.3 mL aqueous ethanol was added. After centrifugation at 14,000 rpm at 4°C, the DNA pellet was washed twice in 0.1 mol/L sodium citrate/10% ethanol. The pellet was then resuspended in 1.4 mL 70% ethanol and left at room temperature for 20 minutes. After centrifugation, the supernatant was discarded and the DNA was air-dried. It was then dissolved in 200 μL 8 mmol/L sodium hydroxide and left overnight at 4°C. The pH was then adjusted to 8.4 with 13.2 μL 0.1 mol/L HEPES. DNA was loaded onto 1% agarose gel and run for 2 hours at 65 V. The DNA ladder was visualized by staining with ethidium bromide and examination under UV light.

Time-lapse photography. Time-lapse video photography of treated and control T3M-4 cells was performed at the Imperial Cancer Research Fund (ICRF) Laboratories (London, UK). Control cells were filmed for 17 hours, and treated cells were filmed for 24 hours. Frames were taken every 60 seconds, and cell numbers were counted by eye at time points of 3, 6, 12, and 24 hours.

RESULTS

Induction of apoptosis in cell lines. JURKAT cells were treated with various doses of camptothecin and then stained with 7AAD. Typical FACS scattergrams of control and treated cells are shown in Fig 1A and B. This shows the three regions defined by 7AAD staining: 7AAD-negative (live cells), 7AAD-dim (apoptotic cells), and 7AAD-bright (late-apoptotic or dead cells). The proportion of apoptotic and dead cells increased in all cases in a dose-dependent manner (Fig 2A). A dose of 20 μg/mL camptothecin was used in subsequent experiments on the basis of the dose response. That the increase in the proportions of cells appearing in the 7AAD-dim and 7AAD-bright regions was caused by apoptotic cell death was shown by the formation of typical DNA fragmentation ladders from treated cells but not from control cells (Fig 3).

TF-1 cells were deprived of GM-CSF for up to 4 days to induce apoptotic cell death; typical scattergrams of control cells and of cells deprived for 4 days are shown in Figs 1C and D. Figure 2B shows the mean ± SEM increase in total nonviable cells with prolonged deprivation. Again, that this was caused by apoptosis was shown by DNA fragmentation ladders produced from deprived cells (Fig 3).

T3M-4 cells required prolonged treatment with camptothecin as compared with that for the JURKAT cells. Representative scattergrams of these cells stained with 7AAD, both before and after 24 hours of incubation with camptothecin, again showed the three regions (Figs 1E and F).

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T3M-4 cells showed a dose-dependent and time-dependent increase in the proportion of apoptotic and dead cells (Fig 2C). In subsequent experiments, cells were incubated with 10 μg/mL camptothecin for 24 hours. No DNA fragmentation ladders were produced, even when 65% of the cells were nonviable (data not shown). However, time-lapse photography of treated T3M-4 cells clearly showed them to be dying by apoptosis (Fig 4).

Sorting of cells by 7AAD-defined regions. The FACStar Plus cell sorter was used to sort all three cell types, according to the regions defined by 7AAD staining. Table 1 shows the purities of the 7AAD-negative, 7AAD-dim, and 7AAD-bright populations before and after cell sorting. Population purity was highest for large, well-defined regions.

Morphology of sorted cells. MGG staining of cytospin preparations of the sorted populations confirmed that cells from each of the three 7AAD-defined regions represented cells with very distinct morphological features. Figure 5 shows sorted JURKAT cells from the three regions. Apoptotic features noted in the 7AAD-dim cells included cell shrinkage, membrane irregularity and cytoplasmic blebbing, nuclear condensation and fragmentation, and, finally, cellular fragmentation. Similar distinct morphological features were observed in the sorted samples of the other cell lines.

TUNEL confirmation of apoptotic cell death. TUNEL was used to confirm further that the cells from the 7AAD-dim region were apoptotic. Incorporation of FITC-dUTP into apoptotic DNA was detected using fluorescence microscopy. Live cells showed no greater fluorescence than negative controls (data not shown). The proportion of TUNEL-positive cells correlated well with the proportion of apoptotic 7AAD-
Fig 2. (A) Dose-response curve of JURKAT cells treated with camptothecin for 4 hours. Results are expressed as mean percentage of 7AAD-dim + 7AAD-bright cells ± SEM of five experiments. (B) Time-response curve of TF-1 cells deprived of GM-CSF for up to 4 days. Results are expressed as mean ± SEM percentage of 7AAD-dim + 7AAD-bright cells of six experiments. (C) Time-response curve of T3M-4 cells treated with various doses of camptothecin.

dim cells in both sorted and unsorted samples. Figure 6 shows TUNEL preparations of sorted TF-1 cells from the three regions; this assay also showed that the 7AAD-bright, or dead, cells had died by apoptosis because their DNA was still labeled in the TUNEL assay (Fig 6C). T3M-4 cells were examined by TUNEL using both labeling with FITC-dUTP and with digoxigenin-peroxidase (ApopTag; see Fig 7): both assays confirmed the apoptotic nature of the 7AAD-dim cells, even though this region was least clearly defined in this cell type (contrast Figs 1B and D with F).

DISCUSSION

In this study, we have validated the method of 7AAD staining in adherent and nonadherent human cell lines, using a combination of other techniques for detecting apoptotic cells. 7AAD staining consistently defined three populations, which were confirmed to represent live, apoptotic, and dead cells after FACS cell sorting. This method was able to measure reproducibly both dose- and time-dependent effects of a variety of apoptosis-inducing stimuli. Furthermore, our results in the T3M-4 cell line emphasize the importance of validating any new method of detecting apoptosis in a given cell system.

There are many methods of detecting apoptotic cells, but all have disadvantages, particularly in the quantitation of cells. The simplest is light microscopy, which can show many of the morphological features characteristic of apoptosis. However, the assessment is subjective and may miss a small and transient apoptotic population. Electron microscopy is much more sensitive but is not readily available, and apoptotic cells thus detected cannot be easily quantitated. Time-lapse photography is believed to be the “gold standard” for identifying and quantitating apoptotic cells, but the process requires expensive equipment and specific expertise; therefore, it is not widely applicable.

DNA fragmentation has been considered for many years...
Fig 4. Time-lapse photographs of T3M-4 cells treated with 10 μg/mL camptothecin and taken (A) at 3 hours, showing features of intact healthy cells, and (B) at 23 hours, showing cell shrinkage and detachment accompanied by extensive membrane blebbing and fragmentation into apoptotic bodies. Each death is rapid, typically 30 minutes from apparent morphological normality to complete fragmentation into apoptotic bodies.

to be the hallmark of apoptosis. However, the presence of a DNA ladder is purely qualitative. Furthermore, large numbers of apoptotic cells may be required to visualize a DNA ladder, typically $5 \times 10^5$ per ladder. The amount of fragmented DNA can be quantitated using densitometry, but this method gives no information about cell number. Also, it is becoming clear that apoptosis can occur (as detected by morphological changes and time-lapse photography) in the absence of a detectable DNA ladder (Kulkarni and McCulloch, Cohen et al, Cohen, and our unpublished observations).

Flow cytometry provides a simple and rapid means of analyzing large numbers of cells and allows for easy quantitation of specific populations. PI staining has been widely used to detect and quantitate apoptotic cells. PI binds to DNA; because the fragmented DNA of apoptotic cells is able to diffuse more rapidly out of the fixed cell, these cells have a lower DNA content and, therefore, have lower mean fluorescence than do live cells, thereby producing the so-called sub-G1 peak on fluorescence histograms. The method requires permeabilization of the cell membrane and, therefore, precludes concurrent immunophenotyping, which would allow the nature of the cells undergoing apoptosis to be further defined. A sub-G1 peak is not observed in all cell systems in which apoptosis is noted to occur by other methods. The sub-G1 peak simply represents cells with reduced DNA content and thus, in addition to apoptotic cells, may be contaminated with cells whose DNA is nonspecifically degraded during necrosis. Other DNA-binding agents have been used to detect apoptosis by flow cytometry, but many, such as ethidium bromide, share the disadvantages of PI. Hoechst 33342 binds preferentially to the DNA of apoptotic, rather than live, cells and, in combination with PI for dead-cell discrimination, provides an efficient means of quantitating the three populations. Dual staining for cell surface markers is also possible, because no permeabilization step

| Table 1. Increase in Population Purity After Cell Sorting |
|-------------|-----------|-----------|
| Population  | Presort % | Postsort %|
| JURKAT      |           |           |
| Live        | 77.3      | 98.8      |
| Apoptotic   | 34.4      | 77.5      |
| Dead        | 15.2      | 31.3      |
| TF-1        |           |           |
| Live        | 70.4      | 94.7      |
| Apoptotic   | 13.6      | 56.9      |
| Dead        | 16.0      | 86.7      |
| T3M-4       |           |           |
| Live        | 33.2      | 95.2      |
| Apoptotic   | 12.2      | 75.3      |

Fig 5. MGG-stained JURKAT cells after sorting (original magnification x400). (A) Cells from live region show typical intact morphological features. (B) Cells from apoptotic region show marked nuclear and cytoplasmic fragmentation and apoptotic body formation. (C) Cells from dead/late-apoptotic region show further nuclear fragmentation and condensation, with more marked cell shrinkage.

Fig 6. TUNEL-labeled TF-1 cells after sorting (original magnification x500). (A) Cells from live region (negative). (B) Cells from apoptotic region, showing bright fluorescent forms, because of incorporation of fluorescein-dUTP into apoptotic bodies. (C) Cells from dead/late-apoptotic region, diffusely positive, showing the cells had died by apoptosis.

Fig 7. ApopTag-staining of T3M-4 cells after sorting (original magnification x400) showing (A) Cells from live region and (B) cells from apoptotic region. Apoptotic cells are detected by direct immunoperoxidase detection of digoxigenin-labeled genomic DNA. Digoxigenin-dUTP is added to 3'OH ends of DNA by TdT. An antidigoxigenin antibody fragment carries a reporter gene (peroxidase) to the reaction site. The localized peroxidase enzyme then catalytically generates an intense signal from chromogenic substrates (positive is brown, see B). The negative counterstain is blue.
is necessary if Hoechst 33342 is the only DNA dye used. However, this dye requires UV laser excitation, which is not widely available in standard flow cytometers.

Activation of the DNA endonuclease and subsequent DNA cleavage results in free 3'-hydroxyl (3'OH) groups in apoptotic DNA. These 3'OH groups can be detected by specific labeling by the enzyme TdT, which will add labeled bases to these 3' ends, i.e., TUNEL. Bases can be labeled with fluorescent compounds (e.g., FITC) and visualized directly by fluorescence microscopy and flow cytometry or with biotin or digoxygenin and can be indirectly identified using secondary labels such as fluoresceinated avidin or peroxidase and chromogenic substrate. This method for identifying and quantitating apoptotic cells is both sensitive and specific, but it is expensive and, unless performed by FACS, requires manual counting, thus limiting both the number of cells that can be assessed and the accuracy of the method.

Staining with 7AAD and analysis by FACS has several advantages over the existing methods outlined above. The staining process is rapid and cheap and, because the apoptotic cells are quantified by the FACS software and not by the eye (as in morphological or TUNEL assessments), large numbers of cells can be examined (minimum of 50,000 in current experiments). Cells are stained before fixing in 2% PF, which has two advantages. Together with the rapidity of the assay, it means that cell morphology and physiology are preserved and the cell membrane is not permeabilized; therefore, concurrent immunophenotyping of cell surface antigens is possible, so that the cell subtypes undergoing apoptosis in heterogeneous populations can be accurately defined. Secondly, fixation in PF after staining means that potentially infectious samples can be handled. The fluorescence is stable for up to 2 hours postfixation because of the high DNA-binding constant and slow dissociation rate of 7AAD, which reduces the rate of leakage of 7AAD out of the positive cells into unstained cells after fixation. We have found that, in samples analyzed within 1 hour, the proportion of cells in each region is unchanged (data not shown).

Any new method of measuring apoptosis needs to be validated in multiple cell systems and by using established methods of detecting apoptotic cells. Schmid et al used human thymocytes and peripheral blood mononuclear cells and confirmed the 7AAD results by FACS cell sorting and morphological examination and by the presence of DNA fragmentation ladders in treated samples. We have extended the experience with 7AAD using three human cell lines, JURKAT, TF-1, and T3M-4, in which apoptosis has been induced by three different means. These cell systems give different patterns of 7AAD staining (see Fig 1; although three distinct regions can be identified in all cases) and differ in their other apoptotic characteristics (e.g., T3M-4 does not show DNA ladder formation). Cell sorting provided pure populations from each region (Table 1), which could then be submitted to the other assays, without excess contamination from other cell types. Because apoptosis is a dynamic process, measurement of the proportion of 7AAD-dim cells gives no indication of the total amount of cell death over time but is related to the rate of apoptosis in a given cell system. Therefore, results were expressed as the sum of the 7AAD-bright and 7AAD-dim cells. Accumulation of cells in the 7AAD-bright region (dead cells) is an important clue to the occurrence of apoptosis in the TF-1 cells (Fig 1D). Indeed, sorted TF-1 cells with high 7AAD fluorescence showed characteristic apoptotic morphology and were positively labeled by TUNEL (Fig 6C), indicating that they had undergone programmed cell death. These cells all stained positively with trypan blue and presumably represent the end-stage of apoptotic cell death, when membrane integrity is lost; whereas those with dim 7AAD fluorescence are still viable (trypan blue-negative) and are at an earlier stage of apoptosis.

The 7AAD assay provided additional evidence that the T3M-4 cells were dying by apoptosis in this system. DNA ladders could not be produced from these cells, even though it was clear from time-lapse photographic study that these cells were undergoing apoptosis (Fig 4). After treatment with camptothecin, there was a dose- and time-dependent increase in the 7AAD-dim + bright population (Fig 2C). However, the dose required to induce apoptosis was larger, and the time course of cell death longer, than that in the JURKAT cells, perhaps reflecting the inherent drug resistance of the T3M-4 cell line. TUNEL assay of this sorted population confirmed that the cells were apoptotic. It has been reported that DNA ladders are not identifiable in some cell systems, perhaps indicating a different mechanism of programmed cell death. This observation underlines the need to evaluate the means of detecting apoptotic cells in each cell system and to compare new protocols critically with established methods.

Unlike the use of other DNA-binding agents, the 7AAD method does not rely on the leakage of low molecular weight DNA fragments from the cell to produce a sub-G1 peak on the fluorescence histogram. Its exact mode of action is unclear, but it is thought to have a different interaction with the membrane of apoptotic cells as compared with that of live cells so that the dye can pass more quickly through the cell membrane, perhaps because of alterations in membrane physiology occurring during apoptosis (e.g., changes in membrane transport pumps). Ormerod et al have shown that altered membrane permeability is responsible for the increased fluorescence of unfixed apoptotic cells stained with Hoechst 33342. 7AAD has the advantage over PI and related compounds in that it is able to identify early apoptotic cells (7AAD-dim), which retain membrane integrity separate from late-apoptotic/dead forms (7AAD-bright), in which membrane integrity has been lost. This is particularly important in systems in which apoptosis is not induced simultaneously in all cells. Accumulation of late apoptotic/dead cells is a major clue to the number of cells that have undergone apoptosis over an extended period of time.

Therefore, 7AAD staining to quantitate live, apoptotic, and late-apoptotic/dead cells by FACS has been evaluated in three diverse human cell lines, and the nature of the cells in the three regions has been verified by cell sorting and, subsequently, by a combination of established methods. This method is quick, simple, reproducible, and cheap and will be a valuable tool in the investigation of
the role of apoptosis in normal physiology and in disease states.

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The use of 7-amino actinomycin D in identifying apoptosis: simplicity of use and broad spectrum of application compared with other techniques

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