

fixed apoptotic cells in the sub-G<sub>0</sub>/G<sub>1</sub> peak of DNA content histogram as a result of loss of DNA fragments out of the cells and because of a reduced DNA “stainability” (Telford et al., 1991, 1992). The latter uses exogenous terminal deoxynucleotidyl transferase (TdT) to label *in situ* the DNA strand breaks with a tracer-dUTP (Gorczyca et al., 1993; Sgonc et al., 1994).

Recent observations have revealed a profound regulatory interrelationship between apoptosis and the cell cycle (Gorczyca et al.). The investigation of this relationship ideally requires techniques that permit concomitant apoptosis detection and cell cycle analysis at a single-cell level.

Two flow cytometric techniques are usually used to investigate the cell cycle: DNA quantification to identify the cell cycle position (Vindelov et al., 1990) and detection of bromodeoxyuridine (BrdU) incorporation to reveal cells going through the S phase (Gratzner, 1982). In this investigation, the development of flow cytometric techniques that permit concomitant detection of apoptosis and cellular DNA content or BrdU content analysis by adapting the apoptosis detection protocol of the Roche Diagnostics *In Situ* Cell Death Detection Kit, Fluorescein is reported.

### 2.1.3 Comparison of two cell death detection methods: *In situ* nick translation and TUNEL

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**Summary:** Apoptosis is a form of regulated cell death characterized by specific morphological changes. These include cell shrinkage, membrane blebbing, chromatin condensation, and cell fragmentation into small apoptotic bodies. At the molecular level, the activation of an endogenous endonuclease results in the fragmentation of cellular DNA into oligosomal length fragments (Martin et al. 1994). These can be readily detected by DNA gel electrophoresis. However, gel electrophoresis does not allow the detection of apoptosis in individual cells.

In contrast, techniques that use enzymatic labeling of DNA strand breaks can provide information regarding apoptosis at a single-cell level. The TdT-mediated dUTP Nick End Labeling (TUNEL) technique uses terminal deoxynucleotidyl transferase (TdT) and allows the labeling of double-stranded DNA breaks (free 3'-OH DNA ends), while the *In Situ* Nick Translation (ISNT) method employs DNA Polymerase I and detects single-stranded DNA breaks. Another advantage of these techniques is that they can be used in combination with cell surface staining or cell cycle analysis. The abilities of the TUNEL and ISNT techniques to detect apoptosis in two types of cells: the IL-3-dependent cell line BAF-3 and freshly isolated CD8<sup>+</sup> lymphocytes from mouse spleen are compared.

### 2.1.4 Fixation of tissue sections for TUNEL combined with staining for thymic epithelial cell marker

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**Summary:** In the thymus, positive and negative selection of thymocytes are important forces that shape the repertoire of mature T lymphocytes in the immune system. In studies on negative selection, it is of great interest to determine whether apoptotic cells reside in thymic cortex or medulla (Surh et al. 1994; Kisielow et al. 1995). Terminal dUTP nick end labeling (TUNEL) is a technique, that is well suited to demonstrate apoptosis *in situ*, and the method may be combined with labeling of other markers. In order to distinguish between thymic cortex and medulla, differential expression of cytokeratin, MHC class II