Chapter 8.2

Cryopreservation of Lymphocytes in Bulk

DOUGLAS M. STRONG

Introduction

Since the early discovery of the cryoprotective properties of glycerol, a great deal of investigation has gone into the determination of the mechanisms of freezing injury (Polge et al., 1949). Very briefly, cells that are cooled too slowly, to below freezing temperatures, are damaged by the resulting increase in salt concentration and cellular shrinkage which occurs as water is removed during the formation of extracellular ice (Lovelock, 1953). Conversely, if cooling is too rapid, a new mechanism is invoked in which shrinkage no longer occurs but the cell is damaged by the formation of intracellular ice, either during freezing or upon thawing (Mazur et al., 1969). Cryoprotectants, such as Me_2SO, reduce the amount of ice present during freezing and reduce solute concentration thus reducing ionic stress (Farrant et al., 1974; Lovelock, 1953; Mazur et al., 1969; Polge et al., 1949). However, these compounds can themselves cause osmotic injury since they are hypertonic and can cause damage during their addition or removal (Strong, 1976). Optimum cooling rates vary from cell type to cell type depending on differences in membrane permeability and intracellular water, which is removed during the dehydration phase of slow cooling and extracellular ice formation. In general, the larger the cell volume, the slower the rate of cooling to allow equilibration of intra- and extra-cellular water during freezing.

Although the primary use of cryopreserved lymphocytes in the histocompatibility laboratory may be as reagents in lymphocytotoxicity assays, where the preservation requirements are less rigid, the use of frozen thawed cells in functional assays or in assays for determination of other cell surface markers, may require closer attention to protocol. Several laboratories have reported that, under certain conditions, selection of lymphoid subsets may occur following freezing and thawing (Farrant et al., 1972; Knight et al., 1972). Furthermore, lymphoid clones and B- or T-LCL have different optimum cooling rates (Strong et al., 1985). Although sophisticated controlled rate freezing devices are not absolutely required for lymphocyte cryopreservation, such equipment usually increases reproducibility and improves recovery (Dubey et al., 1986; Ichino and Ishikawa, 1988). In addition, the handling of cells prior to and following freezing and thawing is at least as important as the freezing itself.

Abbreviations

Me_2SO: Dimethylsulfoxide
DNase: Deoxyribonuclease

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