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 Me₂ SO, 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 membral (Strong, 1976). Optimum cooling rates vary from cell type to cell type depending on differences in membral 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 Ishileawa, 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, SO: Dimethylsulfoxide DNase: Deoxyribonuclease

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ASHI Laboratory Procedure Manual American Society for Histocompatibility and Immunogenetics, Amy B. Hahn, Geoffrey A. Land, Rosemarie M. Strothman, 2000 Handbook of Human Immunology Mary S. Leffell, Albert D. Donnenberg, Noel R. Rose, 1997-04-10 Combining basic explanations of laboratory tests with 115 tables full of reference data and applications the Handbook of Human Immunology provides practicing clinicians with a current complete guide to molecular immunology Introductory chapters overview the molecular basis of immune responses and immunological disorders focusing on the role of cell receptors accessory molecules and cytokines in these processes Emphasis is placed on immunological parameters that are clinically useful The basic principles underlying assays of the immune system are discussed and the book stresses the application and interpretation of immune tests Comprehensive coverage is given to immunoglobulins and their age dependent concentration Cellular immunology is discussed from the perspectives of lymphocyte functional parameters as well as through immunophenotyping of lymphocytes and other leukocytes Both serological and molecular diagnosis of infectious diseases are reviewed The Handbook of Human Immunology contains up to date information on exciting developments in immunogenetics covering the application of T cell receptor genes and the HLA alleles in disease associations and transplantation Manual of Molecular and Clinical Laboratory Immunology Barbara Detrick, John L. Schmitz, Robert G. Hamilton, 2020-07-16 THE authoritative guide for clinical laboratory immunology For over 40 years the Manual of Molecular and Clinical Laboratory Immunology has served as the premier guide for the clinical immunology laboratory From basic serology testing to the present wide range of molecular analyses the Manual has reflected the exponential growth in the field of immunology over the past decades This eighth edition reflects the latest advances and developments in the diagnosis and treatment of patients with infectious and immune mediated disorders The Manual features detailed descriptions of general and specific methodologies placing special focus on the interpretation of laboratory findings and covers the immunology of infectious diseases including specific pathogens as well as the full range of autoimmune and immunodeficiency diseases cancer and transplantation Written to guide the laboratory director the Manual will also appeal to other laboratory scientists especially those working in clinical immunology laboratories and pathologists It is also a useful reference for physicians mid level providers medical students and allied health students with an interest in the role that immunology plays in the clinical laboratory Manual of Molecular and Clinical Lab Immunology Barbara Detrick, Robert G. Hamilton, James D. Folds, 2006-03-10 Introduces new material that reflects the significant advances and developments in the field of clinical laboratory immunology Provides a comprehensive and practical approach to the procedures underlying clinical immunology testing Emphasizes molecular techniques used in the field of laboratory immunology Updates existing chapters and adds significant new material detailing molecular techniques used in the field Presents guidelines for selecting the best procedures for specific situations and discusses alternative procedures Covers

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