Depletion of Human Lymphocytes from Peripheral Blood and Bone Marrow by Affinity Ligands Conjugated to Agarose–Polyacrolein Microsphere Beads

L. WEISS,* S. MARGEL, AND S. SLAVIN

Department of Bone Marrow Transplantation and the Immunobiology Research Laboratory, Hadassah University Hospital, Jerusalem, Israel; and
The Department of Plastic Research, The Weizmann Institute of Science, Rehovot, Israel

Received January 18, 1986; Accepted January 25, 1986

ABSTRACT

Protein-A or goat anti-mouse-Ig (GAMIg) covalently bound to agarose-polyacrolein microsphere beads (APAMB) were employed for the removal of T cells from human peripheral blood leukocytes (PBL) and bone marrow (BM). The cell suspensions were treated with a monoclonal anti-T cell antibody (Leu-1) or monoclonal antilymphocyte antibody (CAMPATH-1) and passed through the conjugated APAMB columns.

Cell separation efficacy was determined by assaying the number and function of T cells in the final cell preparation in comparison with a sample of unseparated cells. The number of cells that form rosettes (E-RFC) with sheep red blood cells (SRBC) in a sample of PBL treated with anti-Leu-1 antibodies and subsequently passed once through GAMIg-conjugated APAMB dropped from a range of 41.5–86.0% to a range of 1.6–13.3%. The in vitro response to concanavalin-A (Con–A) dropped to a range of 0.7–27.2% (GAMIg) and a range of 1.2–21.8% (protein-A column) of the response of untreated PBL. Treatment with

*Author to whom all correspondence and reprint requests should be addressed.
CAMPATH-1 antibody and passage through a protein-A-conjugated APAMB reduced E-RFC from a range of 55.6–57.4% to a range of 3.2–3.9% and abolished the Con-A induced proliferative responsiveness to background levels.

Treatment of BM cells with CAMPATH-1 and passage of the cells through either GAM Ig or protein-A conjugated APAMB columns resulted in reduction of E-RFC from a range of 12.4–17.7% to a range of 0–1% and from a range of 17.7–19% to a range of 1.6–3.2%, respectively. Viability of BM precursors, determined by the CFU-GM assay in semisolid medium, was not affected by these cell separation procedures.

The data suggest that protein-A or GAM Ig-conjugated APAMB columns may be a useful tool for separation of BM cell suspensions into specific cell subsets that can be defined by monoclonal antibodies.

Index Entries: Cell fractionation using immunoadsorbent agarose-polyacrolein beads; bone marrow cells; depletion of T cells from human marrow; monoclonal antilymphocyte antibody (CAMPATH-1); monoclonal anti-T cell antibody (Leu-1); protein A; goal anti-mouse immunoglobulin; colony forming units (CFU-GM).

INTRODUCTION

During the past few years, numerous techniques for cell separation, based mainly on cell size, lectins, density, and charge, have been devised (1–4). One of the simplest and most specific methods for separation of cells into subpopulations according to their unique cell-surface properties is affinity chromatography. Separation of well-defined cell subsets can be accomplished with good purity and viability even when such cells comprise a small fraction of the total cell mixture. Cells can be separated by adsorption on an affinity adsorbent through interactions of cell-surface determinants or receptors with antibodies that can be bound directly or indirectly to a chromatographic column (5–7). In a previous publication, the separation of mouse T and B splenocytes by adsorption onto agarose-polyacrolein microsphere beads (APAMB) was described (8). In the present report, efficient removal of antibody-coated human T lymphocytes by protein-A or goat anti-mouse-Ig (GAM Ig) covalently bound to APAMB is demonstrated. The efficacy of the procedure was established by assaying the capacity of the treated cells to form E-rosettes with sheep red blood cells (SRBC) and by studying proliferative response to concanavalin-A (Con-A) in vitro. Viability of the nonadsorbed bone marrow (BM) cells was unaffected, as determined by colony formation (CFU-GM).

Safe and high effective T cell depletion for prevention of graft versus host disease can be accomplished using a variety of techniques for purging of bone marrow cells ex vivo (9–11). It appears that the procedure presented here can be used for depletion of other defined cell subsets,