INTRODUCTION

It has been shown that the mechanism by which cytolytic T lymphocytes (CTL) lyse target cells can be divided into three successive steps (reviewed in 1): (i) binding of CTL to target cells (adhesion formation), (ii) the action of CTL upon target cells to cause the targets to become irreversibly committed to lyse (programming for lysis, also called the lethal hit), and (iii) actual target cell lysis which occurs independent of continued contact between target cells and CTL. However, the molecular mechanisms by which these processes occur are poorly understood. Studies with monoclonal antibodies which can inhibit lysis in the absence of complement have permitted identification of several molecules which appear to play a role in the cytolytic mechanism (reviewed in 2-6). These include Lyt 2 and its human homologues (2-5), LFA-1 (2-5), T3 (7, 8), and clonotypic molecules which likely represent the T cell.

1Present address: Department of Immunopharmacology, Hoffmann-La Roche, Inc. Nutley, N.J. 07110

2Abbreviations used: CTL, cytolytic T lymphocytes; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfate; Con A, concanavalin A; IL-2, interleukin 2; MLC, mixed leukocyte culture; NK, natural killer; PMA, phorbol myristic acetate; SaCI, Staphylococcus aureus Cowan strain I; SDS-PAGE, polyacrylamide gel electrophoresis run in 0.1% sodium dodecyl sulfate; Tc, cytolytic T cells; Th, helper T cells; TCA, trichloroacetic acid.
receptor (9, 10). Lyt 2 and LFA-1 appear to play a role in the binding of CTL to target cells (3, 5) whereas T3 may serve as a triggering link between the antigen-specific receptor and the lethal hit (6). No molecules have yet been identified which have been shown unambiguously to be involved in the actual delivery of the lethal hit.

The recent demonstration by Harris et al. (11) that liposomes containing membrane components from CTL clones could be used to transfer lytic activity to noncytolytic T and B cell lines suggests that the molecule(s) that mediates specific cytolytic activity resides in or is associated with the cell membranes of CTL. It was, therefore, of interest to compare the membrane-associated proteins of cytolytic and noncytolytic T lymphocytes to determine whether any proteins could be identified which are unique to CTL and thus might play a role in the lytic function of these cells. When we compared the \textsuperscript{35}S-labeled proteins in membrane fractions from cloned lines of alloreactive murine cytolytic (T\textsubscript{c}) and helper (T\textsubscript{H}) T lymphocytes, two proteins were found which were associated with each of three T\textsubscript{c} lines examined but none of four T\textsubscript{H} lines. One of these, p215, was a variant of T200 of higher molecular weight than T200 on T\textsubscript{c}. The other, p24, appeared to be a previously unidentified protein unique to T\textsubscript{c}.

MATERIALS AND METHODS

Generation and maintenance of cloned T cell lines.

Cloned T cell lines were derived by limiting dilution cloning of in vitro sensitized cells as described by Glasebrook and Fitch (12) with minor modifications (13). Primary clonings were performed at a multiplicity of 1 cell per well, and cloned lines were subcloned at a multiplicity of 0.25 cells per well. Established lines were passed in the presence of irradiated DBA/2 splenocytes as feeder cells and lectin-depleted supernatant from cultures of Con A-activated rat splenocytes as a source of interleukin 2 (IL 2) (13).

Characterization of cloned T cell lines

The cytolytic activity of cloned T cells was measured in 4 hr \textsuperscript{51}Cr release assays as previously described (13). Percent specific \textsuperscript{51}Cr release was calculated as \((\% - c)/(100-\% c)\) where \(\%\) represents the percentage of \textsuperscript{51}Cr released in cultures containing both effector and target cells and \(c\) the percentage of \textsuperscript{51}Cr released in control cultures containing target cells alone.