The Lyt-2/3 antigenic complex in the mouse (1) is expressed preferentially on cytolytic T lymphocytes (CTL) and appears to be involved in the recognition of target cells (2,3). In the course of investigating this phenomenon at the clonal level, we observed that individual CTL clones vary greatly in their requirement for Lyt-2/3 molecules in killing. Thus some CTL clones lose cytolytic activity when treated with monoclonal antibodies (MAbs) directed against Lyt-2/3 (4) or with doses of trypsin which selectively cleave the Lyt-2/3 antigenic determinant from the cell surface (5), whereas other clones are resistant to either treatment. Based on these findings, we (6) and others (7,8) have postulated that the role of the Lyt-2/3 molecules may be to facilitate and/or stabilize the interaction between CTL and their target cells. According to this model, the degree to which Lyt-2/3 molecules are required for effective CTL-target cell binding would be inversely related to the intrinsic affinity of the CTL antigen receptor (6).

At the present time, there is little direct evidence pertaining to the functional role of Lyt-2/3 molecules in the cytolytic process. In order to further test the hypothesis that they may be required to enhance low avidity interactions, we have derived a panel of alloreactive CTL clones which vary widely in their dependence upon Lyt-2/3 molecules for cytolysis. These clones were then tested for their ability to lyse thymoma cells in which the density of the appropriate target alloantigen (H-2Kd) could be quantitatively manipulated by exposure to Interferon-γ (IFN) or by pretreatment with monoclonal anti-H-2Kd
antibodies. The results indicate that there is clonal heterogeneity in the ability of CTL to lyse target cells expressing low levels of target H-2K alloantigens. Furthermore, this heterogeneity correlates with the degree of susceptibility of these clones to inhibition by anti-Lyt-2/3 MAbs.

**DERIVATION OF H-2K\(^d\) SPECIFIC CTL CLONES**

CTL clones were obtained either by micromanipulation or by limiting dilution and maintained as described in detail elsewhere (9). For the present experiments, clones were derived from primed spleen cells from C57BL/6 mice immunized 2-6 months previously with P815 (DBA/2) mastocytoma cells and restimulated for 5 days with irradiated DBA/2 spleen cells in secondary mixed leukocyte cultures (MLC) (10). Clones specific for H-2K\(^d\) were selected on the basis of their ability to lyse IT-22 (H-2\(^d\)) mouse fibroblasts transfected with a H-2K\(^d\) genomic clone (kindly provided by Dr S. Kvist, EMBL, Heidelberg) (11).

**EFFECT OF TARGET CELL ANTIGEN DENSITY ON LYSIS BY CTL CLONES**

In order to determine whether the density of surface alloantigen expressed by the target cell influences CTL activity, we made use of our recent discovery that the BALB/c lymphoma ST-4.5 (12) can be stimulated by a factor(s) found in the supernatant of secondary MLC (MLC SN) to increase Class I surface antigen expression. Normally, ST-4.5 cells express very few surface K\(^d\) molecules (approximately 5-20% of the number expressed by P815 cells when compared by flow microfluorometry) and undetectable D\(^d\) or L\(^d\) antigens. After a 48 hour culture in medium supplemented with 10% MLC SN, K\(^d\) expression by ST-4.5 cells increased approximately five-fold and D\(^d\) by twenty-fold, while L\(^d\) remained undetectable (Fig. 1). This effect was apparently specific for H-2 Class I proteins as several other surface antigens (Thy-1, Lyt-2, L3T4 and I-A\(^d\)) did not increase after culture. Furthermore, in agreement with earlier reports (13), pure IFN prepared by recombinant DNA technology (a gift of Dr A. Zlotnik, DNAX, Palo Alto) was found to increase Class I expression in a comparable fashion to MLC SN.

The ability of 8 K\(^d\)-specific CTL clones to lyse normal and MLC SN-induced ST-4.5 cells was then compared. Table I indicates the effect on target (E:T) ratios required for 50% specific lysis of \(^{51}\)Cr-labelled ST-4.5 targets, as well as control P815 targets, by each K\(^d\)-specific CTL clone. Several clones, for example 3,12 and 39, were incapable of efficiently lysing normal ST-4.5 cells but were highly active against ST-4.5 cells whose surface K\(^d\) antigen expression was increased after culture in MLC SN. We do not believe that these results reflect nonspecific