Fine Specificity of Cytotoxic T Lymphocytes Directed Against H-2L^d

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Abstract. The fine specificity of cytotoxic T lymphocytes (CTL) directed against H-2L^d was analyzed by studying the lytic activity of BALB/c H-2^dm2 (H-2L^d loss mutant) anti-BALB/c-H-2^d CTL, generated in secondary mixed lymphocyte culture (MLC) against a panel of target cells of different H-2 haplotypes. Target cells of all H-2 haplotypes tested, except that of the MLC responder, were lysed by anti-L^d CTL, although to a widely varying extent. The genes coding for antigens detected by anti-L^d CTL were mapped to distinct regions in the H-2^d, H-2^dm1, H-2^q, H-2^k, and H-2^b haplotypes. The sequence of lysis intensity against the various H-2 haplotypes and the H-2 regions involved were as follows: L^d, D^kL^b, D^dm1L^d^m1, K^k, D^hL^b, r, p, f, s, C3H.OH (K^d D^kL^b), strong lysis occurring against L^d and weak lysis against H-2^q and C3H.OH.

By monolayer adsorption and cold target inhibition experiments, it was shown that anti-L^d CTL contained a CTL subset directed against a private L^d specificity, hitherto undetected by anti-L^d antibodies. This subset of CTL was separate from the CTL subsets reacting against H-2^q and against the mutant haplotype H-2^dm1. The reactions against the latter two haplotypes were also mediated by separate CTL subsets. It is concluded that the L^d molecule, to a varying extent, shares target antigens for CTL with K- and/or D-end H-2 molecules of all haplotypes tested. These antigens are detected by multiple subsets of anti-L^d CTL. One CTL subset is directed against a target structure unique for L^d (L^d private specificity).

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Introduction

The H-2L molecule is encoded by a gene closely linked to the H-2D locus (Lemonnier et al. 1975, Neauport-Sautès et al. 1977, McKenzie et al. 1977, Hansen et al. 1977b, Démant and Neauport-Sautès 1978, Hansen and Sachs 1978) and resembles the H-2K and H-2D molecules; it is a glycoprotein of approximately 45,000 daltons molecular weight (Neauport-Sautès et al. 1977, Hansen et al. 1977a, Hansen and Sachs 1978) and shows amino-acid homology with the K and D molecules (Coligan et al. 1980). The analysis of alloantigens specified by H-2L and of the role of H-2L in associative recognition of viral antigens has been facilitated by the availability of an L-locus loss mutant, BALB/c-H-2^am2 (Melvold and Kohn 1976, McKenzie et al. 1977, Blanden et al. 1977, Hansen and Sachs 1978, Hansen and Levy 1978, Levy et al. 1978, Iványi et al. 1979). Analysis of BALB/c-H-2^am2 antisera (anti-L^d sera) has shown that the L^d molecule expresses public antigens of the H-2.28 family (Démant and Neauport-Sautès 1978) and the new public antigens H-2.64, 65 (Hansen and Sachs 1978) and H-2.73, 74, 75 (Huang et al. 1979). So far, no private L^d specificity has been identified by serology. Cytotoxic T lymphocytes (CTL) generated in secondary mixed lymphocyte culture (MLC) against L^d, however, recognize an antigen that is unique for L^d (L^d private specificity). This is evident from the following findings: (1) H-2^am2 anti-H-2^d CTL show strongest lytic capacity for targets expressing L^d, while all other targets are lysed less strongly (Melief et al. 1979, Levy and Hansen 1980). (2) H-2^am2 anti-H-2^d CTL show a strong cross-reaction against B10.AKM (H-2^m) target cells which bear all serologically recognized antigens of L^d. Nevertheless, (B10.AKM × BALB/c-H-2^am2)F_1 hybrid mice mounted a strong CTL response to BALB/c-H-2^d cells, showing that H-2^m could not complement H-2^am2 in this assay (Levy and Hansen 1980). (3) Skin-graft studies have shown that none of the haplotypes tested (H-2^b, H-2^k, H-2^p, H-2^am1) could complement H-2^am2 (McKenzie et al. 1977, Morgan et al. 1978). Although this is indirect evidence, it supports the CML data.

In this report we confirm and extend the notion of a private L^d specificity detected by H-2^am2 anti-H-2^d (anti-L^d) CTL, using monolayer absorption and cold target inhibition techniques. The results indicate that anti-L^d CTL contain a unique CTL subset reactive only with L^d and separate from the subsets reacting against H-2^q and H-2^am1. The reactions against the latter two haplotypes were also mediated by separate CTL subsets.

Reactivity of anti-L^d CTL with K^k molecules (Hansen et al. 1979) was confirmed. In addition, we could map the cross-reactivity of anti-L^d CTL against H-2^q to the D end of this haplotype. In general, cross-reactivity of anti-L^d CTL against other haplotypes was extensive and showed a wide range of intensity with lowest, but still demonstrable, lysis against C3H.OH (H-2K^d D^k L^k) and B10.S or A. SW (H-2^o) target cells.

Materials and Methods

Mice. All mice were bred by full sib-sib mating at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB). Breeding stock of parental BALB/cKh and mutant BALB/c-H-2^am2