

THE Lyt-2 MOLECULE RECOGNIZES RESIDUES IN  
THE CLASS I  $\alpha$ 3 DOMAIN IN ALLOGENEIC  
CYTOTOXIC T CELL RESPONSES

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T cell recognition of foreign class I antigens involves an interaction between the TCR  $\alpha$  and  $\beta$  chains and the polymorphic amino acids of the  $\alpha$ 1 and  $\alpha$ 2 domains of the class I antigen. The three-dimensional structure of a human class I antigen has recently been determined and suggests that residues from the  $\alpha$ 1 and  $\alpha$ 2 domains that are recognized by the TCR are clustered on top of the molecule and form the recognition site for processed antigens (1, 2). Residues from the  $\alpha$ 3 domain apparently do not contribute to this recognition site, which is consistent with previous studies demonstrating that CTL do not recognize polymorphic residues in the  $\alpha$ 3 domain (3-5).

There is considerable evidence suggesting that in addition to the TCR, several "accessory" molecules on the T cell surface facilitate interaction with a target cell. Among these accessory molecules are Lyt-2 (CD8), which facilitates the reactivity of T cells with class I MHC molecules, and L3T4 (CD4), which performs a similar role for class II-reactive T cells. The experimental observations that implicate these accessory molecules in T cell recognition come from systems in which antibodies to Lyt-2 or L3T4 inhibit the function of class I- or class II-reactive T cells, respectively (6-8). More recently it has been demonstrated that transfection of the Lyt-2/CD8 gene, as well as TCR- $\alpha$  and - $\beta$  genes, is necessary to confer reactivity to class I determinants in a recipient cell (9, 10). It has been suggested that the Lyt-2 molecule binds to a determinant expressed on all class I molecules on the target cell, thereby facilitating interaction between the effector and target cells (11, 12). This determinant would be conserved, or monomorphic; however, the nature of this determinant has yet to be defined.

The ability of mAb to the class I  $\alpha$ 3 domain to block CTL function (3) as well as the characterization of a somatic cell mutant that expresses an alteration in the  $\alpha$ 3 domain of H-2D<sup>d</sup> (13) have raised the possibility that residues in the  $\alpha$ 3 domain of class I molecules are recognized by CTL. The nature of the mutation in the H-2D<sup>d</sup> gene has been identified and reproduced in a cloned gene by oligonucleotide mutagenesis (14). Cells transfected with this mutant gene are not killed by anti-D<sup>d</sup>-reactive CTL generated in a primary in vitro response. In this paper, we pro-

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vide evidence that residues in the  $\alpha 3$  domain of class I molecules on the target cell contribute to a conserved determinant that is recognized by the Lyt-2 molecule on CTL. Several lines of evidence support this conclusion: (a) mAbs to determinants in the  $\alpha 3$  domain of H-2L<sup>d</sup> or H-2D<sup>d</sup> inhibit the generation of primary CTL in vitro but do not inhibit the generation of and only partially inhibit the function of secondary CTL; (b) secondary CTL populations generated in the presence of antibodies specific for the  $\alpha 3$  domain of the stimulating antigen, in contrast to primary or secondary CTL generated in the absence of mAb, are only weakly inhibited by antibody to Lyt-2; (c) treatment of mice with graft-specific antibodies to both  $\alpha 3$ , as well as  $\alpha 1/\alpha 2$  domain determinants is necessary to enhance the survival of skin grafts; (d) target cells expressing a mutant H-2D<sup>d</sup> molecule that possesses a single amino acid substitution in the  $\alpha 3$  domain are not killed by CTL generated in a primary in vitro response, but are killed by the Lyt-2-independent CTL population generated by secondary stimulation in the presence of antibody to the  $\alpha 3$  domain of H-2D<sup>d</sup>; and (e) cells expressing the  $\alpha 3$  mutant D<sup>d</sup> molecule fail to elicit a primary in vitro cytotoxic response. In total, these results identify residues in the class I  $\alpha 3$  domain that are involved in Lyt-2 recognition and furthermore suggest that mAbs to determinants in the  $\alpha 3$  domain can be used in vivo and in vitro to block this Lyt-2-dependent recognition.

### Materials and Methods

*Mice.* BALB/cKh (K<sup>d</sup>, D<sup>d</sup>, L<sup>d</sup>), BALB/c-H-2<sup>dm2</sup> (K<sup>d</sup>, D<sup>d</sup>), C3H (K<sup>k</sup>, D<sup>k</sup>), and (C3H.OL (K<sup>d</sup>, D<sup>k</sup>) × BALB.K (K<sup>k</sup>, D<sup>k</sup>))F<sub>1</sub> were bred in the animal facility of Dr. Donald C. Shreffler, Washington University School of Medicine, St. Louis, MO.

*Antibodies.* The mAbs used have been previously described. Briefly, antibody 30-5-7 was derived in dm2 mice and was shown to recognize an epitope in the  $\alpha 1/\alpha 2$  domain of L<sup>d</sup>, whereas antibody 28-14-8 was derived from C3H mice and was shown to recognize the  $\alpha 3$  domain of L<sup>d</sup> and several other D region-encoded molecules (15, 16). Antibodies 34-5-8 and 34-2-12 were both derived from C3H mice and were shown to recognize the  $\alpha 1/\alpha 2$  and  $\alpha 3$  domains of D<sup>d</sup>, respectively (16, 17). Antibody 3-83 was derived from BALB/c and recognizes H-2<sup>k</sup> antigens (18). All five of the above mAbs have the  $\gamma 2a$  isotype and when grown as an ascites contained a titer of antibody of ~30,000, as measured by dye-exclusion cytotoxicity (data not shown). They also have comparable affinities based on analysis by flow microfluorometry (FMF).<sup>1</sup> The mAbs 53-6.7 and 53-7.3 were both derived from the rat, have the  $\gamma 2a$  isotype, and recognize Lyt-2 and Lyt-1, respectively (19). All antibodies were analyzed by FMF on splenic T cells and/or target cell lines and were titered in CTL assays before use in the experiments reported here.

*Skin Grafting.* Skin grafts were performed as described previously (20). Full thickness donor tail skin grafts were trimmed to ~0.5 × 1.0 cm. Appropriately sized graft beds were prepared on the recipient's dorsal thorax by carefully removing skin with scissors without disturbing the panniculus carnosus. All grafts were male to male. The transplanted tissues were protected with a gauze dressing and a plaster bandage for 7 d. Grafts were scored as rejected when <10% of the donor tissue was viable by gross inspection.

*Culture of Cell Lines.* All of the cell lines used as targets were grown in DME (high glucose) supplemented with L-glutamine, pyruvate, and 10% FCS. P815 is a mouse mastocytoma isolated from a DBA/2 mouse. R8.15.28 (D<sup>d</sup> glu) and L.D<sup>d</sup>.28 express the transfected wild-type D<sup>d</sup> gene and R8.15.29 (D<sup>d</sup> lys) and L.D<sup>d</sup>.29 express the transfected  $\alpha 3$  mutant D<sup>d</sup> gene as previously described (14). The R8.15 cell line into which the genes were transfected was iso-

<sup>1</sup> Abbreviations used in this paper: dm2, L<sup>d</sup> loss mutant mouse strain BALB/c-H-2<sup>dm2</sup>; FMF, flow microfluorometry.

lated from R8 (H-2<sup>d</sup> × H-2<sup>b</sup>)F<sub>1</sub> by immunoselection and does not possess H-2<sup>d</sup> genes (21). The L.D<sup>d</sup>.28 and L.D<sup>d</sup>.29 cell lines were produced by transfection into L cells, a fibroblast cell line originating in C3H (H-2<sup>k</sup>) mice. The R8.15 transfected cell lines were cultured in the presence of 400 µg/ml G-418 sulfate antibiotic (Sigma Chemical Co., St. Louis, MO) and the L cell-transfected cell lines were cultured in the presence of 200 µg/ml G-418 sulfate antibiotic. Cells were used for targets during log phase of growth.

**Fluorescence Labeling of Cells and Analysis by FME.** For fluorescence analysis, cells were washed, labeled, and analyzed in HBSS lacking phenol red but containing 0.2% BSA and 0.1% sodium azide (FME medium).  $4 \times 10^5$  cells were placed in each well of a round-bottomed microtiter plate (Flow Laboratories, Inc., McLean, VA), washed two times, and incubated for 30 min at 4°C in the presence of a saturating concentration of mAb. The cells were washed two times and resuspended in a saturating concentration of fluorescein-conjugated, affinity-purified F(ab')<sub>2</sub> fragment of goat anti-mouse IgG, Fc specific, or of goat anti-rat IgG (CooperBiomedical, Inc., Malvern, PA). The cells were incubated for 30 min at 4°C then washed three times and resuspended in FME medium containing 10 µg/ml propidium iodide, used to exclude dead (red fluorescent) cells from analysis.

Cells were analyzed on a FACS IV (Becton & Dickinson Co., Mountain View, CA) equipped with an argon ion laser tuned to 488 nm and operating at 300 mW of power. Fluorescence histograms were generated with logarithmic amplification of fluorescence emitted by single viable cells. Each sample analyzed comprised a minimum of  $5 \times 10^4$  cells. Cells labeled with only the fluorescein-conjugated antibody were always included as controls.

**Generation of H-2L<sup>d</sup>- or H-2D<sup>d</sup>-specific CTL.** Responding spleen cells ( $7.5 \times 10^6$ ) were cocultured with stimulating spleen cells ( $3.5 \times 10^6$ , 2,000 rad), L cells ( $5 \times 10^4$ , 10,000 rad) or medium alone in 24-well Linbro trays (Flow Laboratories, Inc.) containing 2 ml RPMI 1640 medium (Mediatech, Washington, DC) supplemented with L-glutamine, sodium pyruvate, nonessential amino acids, penicillin, streptomycin,  $5 \times 10^{-5}$  M β-mercaptoethanol, and 10% FCS (HyClone Laboratories, Logan, UT). After 5 d at 37°C in the presence of 5% CO<sub>2</sub>, effector cells were harvested, washed, and resuspended in RPMI 1640 medium supplemented with 10% FCS (RPMI-FCS). For those experiments that included mAbs during the 5-d sensitization,  $10^8$  stimulator spleen cells were preincubated with 500 µl of mAb ascites (1:10 dilution in culture medium) for 30 min on ice. They were washed and resuspended in fresh medium containing the appropriate antibody before addition to the culture wells. The final concentration of antibody in the culture was 1%.

**<sup>51</sup>Cr-release Assay.**  $3 \times 10^6$  target cells were labeled with 150–300 µCi of <sup>51</sup>Cr (Na<sup>51</sup>CrO<sub>4</sub>, 10–25 mCi/ml; Amersham Corp., Arlington Heights, IL) in 300 µl of RPMI-FCS for 45 min at 37°C in 5% CO<sub>2</sub>. Cells were washed twice and  $10^4$  cells were added to the wells of round-bottomed microtiter plates. For antibody blocking studies, 50 µl of antibody was preincubated with 50 µl of target cells or 100 µl of effector cells or medium for 20 min at 37°C. The remaining cells were added to a final volume of 200 µl per well, the plates were spun at 50 g for 1 min and incubated in a humidified atmosphere at 37°C, 5% CO<sub>2</sub>. At the end of 4 h the plates were spun at 800 g for 5 min and 100 µl of supernatant was counted in a Searle automatic gamma counter (Searle Analytic, Des Plaines, IL). The mean of triplicate samples was calculated and percent <sup>51</sup>Cr release was determined according to the following equation: Percent <sup>51</sup>Cr release =  $100 \times \frac{[(\text{experimental } ^{51}\text{Cr release} - \text{control } ^{51}\text{Cr release})]}{(\text{maximum } ^{51}\text{Cr release} - \text{control } ^{51}\text{Cr release})}$ , where experimental <sup>51</sup>Cr release represents counts from target cells mixed with effector cells, control <sup>51</sup>Cr release represents counts from target cells incubated in medium alone (spontaneous release), and maximum <sup>51</sup>Cr release represents counts from target cells exposed to 5% Triton-X100. For the data presented in this paper the SEM percent specific lysis was <5% of the value of the mean.

## Results

**Antibody to the α3 Domain of H-2L<sup>d</sup> Inhibits Lysis by Primary, but not Secondary, CTL Populations.** To delineate the involvement of the different domains of class I molecules in CTL recognition, we examined the ability of antibodies specific for either the

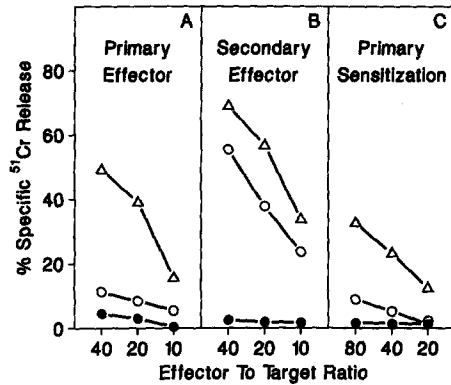


FIGURE 1. Effect of mAbs to the  $\alpha 1/\alpha 2$  and  $\alpha 3$  domains of  $L^d$  on the sensitization and effector function of  $L^d$ -specific CTL. mAbs 28-14-8 (O) or 30-5-7 (●) were added to the CTL assays of primary (A) or secondary (B) anti- $L^d$  CTL, or were included during the 5-d sensitization of dm2 anti BALB/c in vitro primary response (C). ( $\Delta$ ) Control response. The final antibody concentration was 1% in all cases. The target was P815. Each figure represents a separate experiment.

$\alpha 1/\alpha 2$  or the  $\alpha 3$  domain to inhibit lysis by  $L^d$ -specific CTL. Spleen cells from BALB/c-H-2<sup>dm2</sup> (dm2) mutant mice that have undergone a deletion of the  $L^d$  gene (22) were stimulated in vitro with spleen cells from BALB/c mice. Antibodies to either the  $\alpha 1/\alpha 2$  (30-5-7) or the  $\alpha 3$  (28-14-8) domain strongly inhibited lysis of target cells by these  $L^d$ -specific CTL derived by primary in vitro stimulation (Fig. 1 A). We next examined whether these same antibodies would inhibit anti- $L^d$  CTL generated in a secondary response. Dm2 mice were primed in vivo by grafting of BALB/c skin. 1-2 wk after rejection, spleen cells from these mice were restimulated in vitro. Although we refer to this population as secondary CTL, it is possible that some unprimed T cells are also stimulated and therefore some primary CTL may be present. Lysis of  $L^d$ -positive targets by this secondary CTL population was inhibited by the 30-5-7, but not the 28-14-8 antibody (Fig. 1 B). There are several explanations for the inhibition of CTL function by antibodies including: (a) binding of the antibody creates a steric hindrance for CTL recognition and/or killing, (b) binding of the antibody alters the conformation of allodeterminants recognized by CTL, or (c) the antibody binds to residues that are necessary for CTL recognition. While it is usually difficult to distinguish between these alternatives, the observation that 28-14-8 did not inhibit the activity of secondary CTL indicated that this antibody did not cause

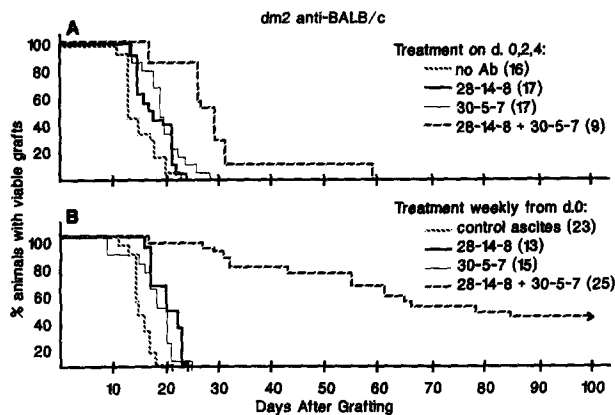


FIGURE 2. Survival of BALB/c skin grafts on dm2 recipients ( $L^d$  disparate) given either no antibody, 0.2 ml of mAb 28-14-8, 0.2 ml of mAb 30-5-7, or a combination of 0.1 ml of 28-14-8 and 0.1 ml of 30-5-7 for the times indicated starting with the day of grafting. The control mAb ascites used was 3-83 (anti-H-2<sup>k</sup>). The numbers in parentheses represent the number of animals in each treatment group.

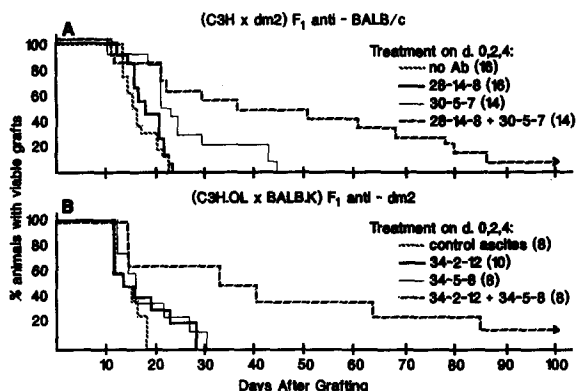


FIGURE 3. Survival of  $L^d$ -disparate (A) or  $D^d$ -disparate (B) skin grafts in mice given antibodies to MHC class I determinants. Animals received either 0.2 ml of one antibody or 0.1 ml of both antibodies at the times indicated starting with the day of grafting. The numbers in parentheses represent the number of animals in each treatment group.

conformational changes in allodeterminants, nor a steric hindrance, for secondary CTL activity. This finding also raised the possibility that the inhibition by 28-14-8 of primary CTL activity was because this antibody bound a determinant that was recognized by primary CTL, but not by secondary CTL.

**Antibodies to Either the  $\alpha 1/\alpha 2$  or the  $\alpha 3$  Domain of  $H-2L^d$  Inhibit the Generation of Primary CTL *In Vitro*.** Having established that there was a difference between primary and secondary anti- $L^d$  CTL in the susceptibility to inhibition by the  $\alpha 3$  domain antibody when added during the effector phase, we next examined the effect of antibodies added during the sensitization phase. Addition of the 28-14-8 ( $\alpha 3$  domain) or the 30-5-7 ( $\alpha 1/\alpha 2$  domain) antibodies during the 5-d *in vitro* sensitization abrogated the generation of  $L^d$ -specific primary CTL (Fig. 1 C). To examine whether these antibodies would also inhibit the generation of secondary CTL we tested the effect of antibodies added during *in vivo* priming.

**Treatment with a Combination of Antibodies to  $\alpha 1/\alpha 2$  and  $\alpha 3$  Epitopes of  $L^d$  has a Synergistic Effect on Skin Graft Enhancement.** Priming of CTL *in vivo* can be achieved either by the injection of allogeneic cell suspensions or by performing an allograft. We decided to perform allografting as it allowed us to monitor the effect of antibodies on the priming of CTL, not only through the subsequent analysis of primed CTL populations, but also by the impact of antibodies on the survival of the graft. The injection of antibodies to class II MHC molecules expressed on an allograft can be very effective in enhancing skin graft survival (23). As the antibodies to  $H-2L^d$  inhibited the generation and the activity of CTL *in vitro*, a similar effect *in vivo* would enhance the survival of  $H-2L^d$ -disparate skin grafts. To confine the disparity to  $H-2L^d$ , dm2 mice received a graft from BALB/c mice. Some of the skin-grafted animals were injected with antibody to either the  $\alpha 1/\alpha 2$  domains (30-5-7), or the  $\alpha 3$  domain (28-14-8) of  $L^d$  or with both antibodies. Animals that received either of the two antibodies on days 0, 2, and 4 showed a slight enhancement of skin graft survival compared with animals that received no antibody or an irrelevant antibody (anti  $H-2K^k$ ) on days 0, 2, and 4 (Fig. 2 A). Graft survival was enhanced more when animals received both antibodies together on days 0, 2, and 4 (Fig. 2 A). This finding is consistent with a recent report by Lems et al. (24). Since both antibodies were found to have a comparable *in vivo* half-life of  $\sim 3$  wk (cytotoxicity data not shown), a regimen of weekly antibody injections was also tested. When the injections of anti-

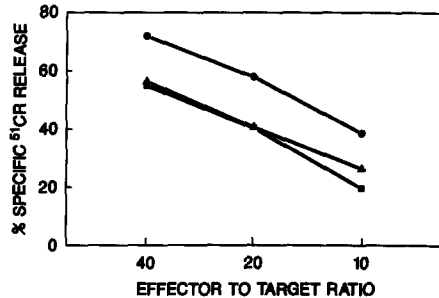


FIGURE 4. In vitro L<sup>d</sup>-specific CTL responses after in vivo skin graft priming of animals treated with graft-specific antibodies. Dm2 mice received 28-14-8 in vivo (●), 30-5-7 in vivo (▲), or 28-14-8 + 30-5-7 in vivo (■) at the time of grafting with BALB/c skin. Spleen cells from each group were restimulated in vitro with BALB/c spleen cells. P815 was used as the target cell and the spontaneous release was 5.4%. Unstimulated cells from each group were included and their L<sup>d</sup>-specific lysis was zero.

body were given weekly, dramatic synergism between the two antibodies was observed (Fig. 2 B). About 40% (11/25) of the mice that received both antibodies had viable tissue grafts after 100 d. In contrast, mice that received only one of the H-2L<sup>d</sup> antibodies rejected their grafts before 24 d. A similar enhancement of graft survival by treatment with both H-2L<sup>d</sup> antibodies was also seen in (C3H × dm2)F<sub>1</sub> mice grafted with BALB/c skin. In this experiment the injections of antibody were given on days 0, 2, and 4 and the injection of both antibodies together enhanced the survival of the graft in these F<sub>1</sub> mice even more than similarly timed injections given to dm2 mice (Figs. 3 A and 2 A). The finding that enhancement appears easier to obtain in the F<sub>1</sub> mice as compared with dm2 mice could be accounted for by either (a) a weaker anti-L<sup>d</sup> response in the F<sub>1</sub> due to elimination of H-2<sup>k</sup>-crossreactive CTL clones or (b) the matching of Ig allotype/idiotypic in the F<sub>1</sub> since 28-14-8 is a C3H-derived antibody. In addition to examining the effect of H-2L<sup>d</sup> antibodies on the survival of H-2L<sup>d</sup>-disparate grafts we also investigated whether a combination of antibodies to  $\alpha 1/\alpha 2$  and  $\alpha 3$  H-2D<sup>d</sup> determinants could enhance the survival of H-2D<sup>d</sup>-disparate grafts.

To confine the disparity to H-2D<sup>d</sup> determinants, (C3H.OL × BALB.K)F<sub>1</sub> recipients were grafted with dm2 skin. Some of these animals received either the 34-2-12 antibody (specific for  $\alpha 3$  of D<sup>d</sup>), or the 34-5-8 antibody (specific for  $\alpha 1/\alpha 2$  of D<sup>d</sup>), or a combination of the two antibodies on days 0, 2 and 4. Antibody to either the  $\alpha 1/\alpha 2$  or  $\alpha 3$  domains alone caused only slight enhancement of skin graft survival, whereas a combination of the two antibodies had a synergistic effect and resulted in prolonged graft survival (Fig. 3 B). This synergism between  $\alpha 1/\alpha 2$  and  $\alpha 3$  domain antibodies in the prolongation of either H-2L<sup>d</sup> or H-2D<sup>d</sup>-disparate skin grafts demonstrated that regimens using combinations of antibodies to class I can enhance class I-disparate skin grafts. This finding is in contrast to previous studies in which antibodies to class II but not class I antigens enhanced allograft survival (23). In addition, these data raise the possibility that recognition of both the  $\alpha 1/\alpha 2$  and  $\alpha 3$  domains of the class I molecule are involved in allograft rejection.

*In Vitro L<sup>d</sup>-specific CTL Responses of Mice Injected with Antibodies to H-2L<sup>d</sup>.* To delineate the mechanism of the synergistic effect of these antibodies on skin graft enhancement, antibody-treated mice were tested for the generation of CTL in vitro. Dm2 mice that were grafted with BALB/c skin (anti-L<sup>d</sup>) and had received either no antibody, one antibody, or both antibodies (28-14-8 and 30-5-7) were tested for their ability to generate L<sup>d</sup>-specific CTL in vitro. From animals treated with a single antibody, cultures were initiated at least 3 wk after graft rejection, and from animals

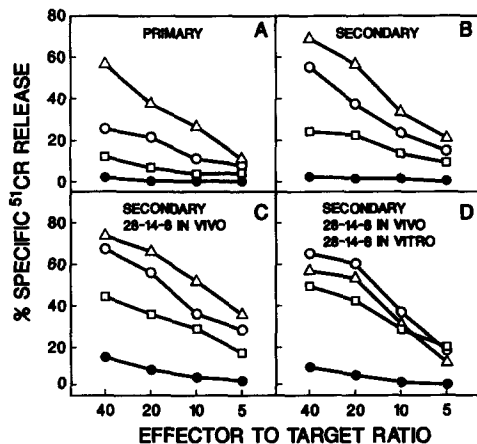


FIGURE 5. Antibody blocking of L<sup>d</sup>-specific (dm2 anti-BALB/c) CTL generated from primary and secondary in vitro cultures. The secondary cultures were generated after skin graft priming (C). Some of the skin-grafted animals received 28-14-8 on the day of grafting and weekly until rejection (C) and 28-14-8 was also included in some of the cultures from the antibody-treated animals (D). The antibodies included in the CTL assay were 3-83-negative control ( $\Delta$ ), 28-14-8 (O), 30-5-7 ( $\bullet$ ), and 53-6.7 ( $\square$ ). Antibody 53-7.3 was also included as a negative control and lysis in the presence of this antibody was not different from lysis in the presence of the 3-83 antibody control. Unstimulated control cells from each group were included and lysis was zero. Spontaneous release from the P815 target cells was 10.5%.

that received both antibodies cultures were initiated from mice that possessed a viable graft at least 5 wk after grafting.

Spleen cells from mice treated with either 28-14-8 or 30-5-7, or both antibodies together, generated anti-L<sup>d</sup> CTL after in vitro stimulation. The CTL response of skin-grafted animals that received 28-14-8 in vivo (Fig. 4) was indistinguishable from that of skin-grafted animals that did not receive any antibody (data not shown), but was greater than the response from animals that received either 30-5-7 alone or together with 28-14-8 (Fig. 4). It is interesting that animals treated with both antibodies and showing no visible sign of graft rejection were able to generate an in vitro anti-L<sup>d</sup> CTL response. In addition, discontinuation of the weekly antibody treatments leads to rejection of the grafts (data not shown).

*In Vivo and In Vitro Treatment with  $\alpha 3$  Domain-specific mAb Enhances the Generation of L<sup>yt</sup>-2-independent CTL.* The data presented in Figs. 1-4 suggest that antibodies to either  $\alpha 1/\alpha 2$  or  $\alpha 3$  H-2L<sup>d</sup> determinants can block the in vitro generation of alloreactive CTL from nonimmune mice; however, both antibodies are required to prolong skin graft survival in vivo. In addition, spleen cells from skin-grafted mice treated with either, or both, of the H-2L<sup>d</sup> antibodies, could be stimulated in vitro to generate anti-L<sup>d</sup> CTL. We therefore examined whether the addition of H-2L<sup>d</sup> antibodies in vitro would inhibit the generation of anti-L<sup>d</sup> CTL from antibody-treated, skin-grafted mice. As shown in Figs. 5 (A and B) and 1 (A and B), antibody to the  $\alpha 1/\alpha 2$  domains completely inhibits lysis by both primary and secondary CTL generated in the absence of antibody. In contrast, the  $\alpha 3$  domain antibody did not inhibit primary CTL (Figs. 5 B and 1 B) as effectively as it inhibited the activity of primary CTL (Figs. 5 A and 1 A). Secondary CTL from mice injected with the  $\alpha 3$  domain antibody at the time of grafting were even less inhibited by 28-14-8 (Fig. 5 C) than secondary CTL from mice that were not injected with antibody (Fig. 5 B). The activity of secondary CTL generated in the presence of 28-14-8 both in vivo and in vitro was essentially noninhibitable by the addition of 28-14-8 (Fig. 5 D). It is also interesting that the secondary CTL generated in the presence of 28-14-8 in vivo were not totally inhibitable with the  $\alpha 1/\alpha 2$  domain antibody 30-5-7. This suggested either that these CTL have a higher affinity than those derived from animals

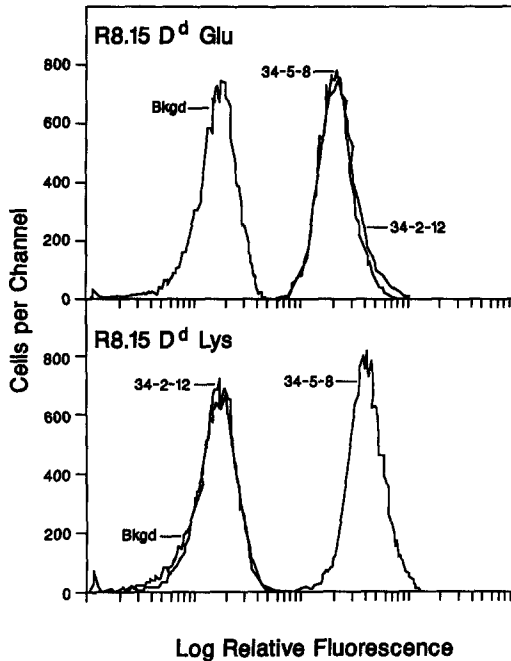


FIGURE 6. Expression of H-2D<sup>d</sup>-specific determinants defined by mAb 34-5-8 ( $\alpha 1/\alpha 2$  domain specific) and mAb 34-2-12 ( $\alpha 3$  domain specific) on cells transfected with the wild-type D<sup>d</sup> gene (R8.15 D<sup>d</sup> glu) and cells transfected with the  $\alpha 3$  mutant D<sup>d</sup> gene (R8.15 D<sup>d</sup> lys). Antibody-treated cells were developed with F(ab')<sub>2</sub> fragment of goat anti-mouse IgG, Fc specific. The background histograms represent fluorescence emitted from cells treated only with the fluorescein-conjugated developing antibody.

not treated with antibody, or alternatively, that a minor CTL population can recognize L<sup>d</sup> in the presence of bound 30-5-7. The effect of Lyt-2 antibody on the lysis by these CTL populations was also examined. It was found that although antibody to Lyt-2 almost totally inhibited primary CTL (Fig. 5 A), the antibody had less inhibitory effect on secondary CTL (Fig. 5 B) and had very little effect on secondary CTL generated in the presence of 28-14-8 in vivo and in vitro (Fig. 5 D). These results suggested that the addition of 28-14-8 antibody favored the generation of Lyt-2-independent, over Lyt-2-dependent, anti-L<sup>d</sup> CTL.

*Cells Expressing a Mutation in the  $\alpha 3$  Domain Are Killed by Lyt-2-independent Secondary CTL but not Lyt-2-dependent Primary CTL.* The observation that antibodies to the  $\alpha 3$  domain enhanced the generation of Lyt-2-independent CTL raised the possibility that the residues of the  $\alpha 3$  domain bound by the 28-14-8 antibody also contributed to the determinant recognized by the Lyt-2 molecule of CTL. If so, then Lyt-2-dependent CTL could not associate with target cells with antibody bound to the  $\alpha 3$  domain because the antibody would prevent binding by the Lyt-2 molecule. In contrast, Lyt-2-independent CTL would not be inhibited by  $\alpha 3$  domain antibody, and in addition, could kill target cells that have lost the determinant recognized by the Lyt-2 molecule.

Recently it was demonstrated that substitution of lysine for glutamic acid at residue 227 in the  $\alpha 3$  domain of H-2D<sup>d</sup> abrogates binding of the H-2D<sup>d</sup>  $\alpha 3$  domain specific antibody, 34-2-12, and lysis by primary anti-D<sup>d</sup> CTL (13, 14). FMF analysis demonstrated that there was no quantitative difference in the level of H-2D<sup>d</sup> expression between cells transfected with the mutant (D<sup>d</sup> lys) or the wild-type (D<sup>d</sup> glu) H-2D<sup>d</sup> gene, as both cells bound an equivalent amount of the  $\alpha 1/\alpha 2$  domain



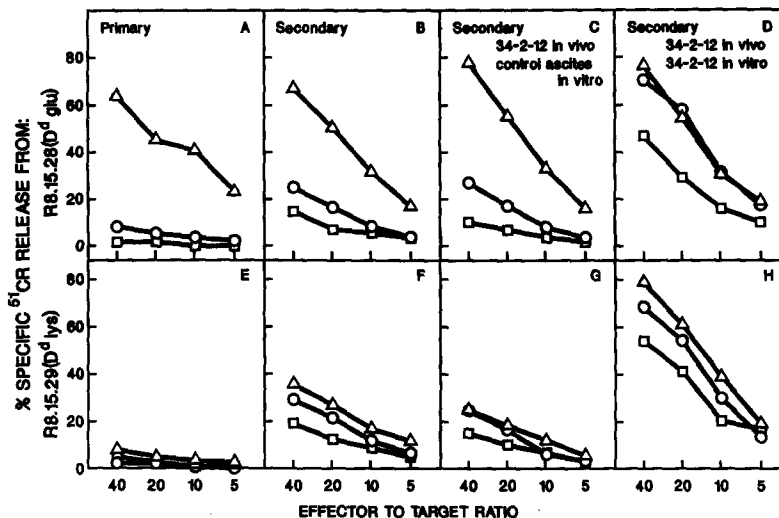


FIGURE 7. Antibody blocking of  $D^d$ -specific,  $(C3H.OL \times BALB.K)F_1$  anti-dm2, CTL generated from primary and secondary in vitro cultures and tested on the cells transfected with the wild-type or mutant  $H-2D^d$  gene. The secondary cultures were generated after in vivo skin graft priming animals (B and F), some of which received 34-2-12 in vivo (C and G). Some of the cultures from the antibody-treated animals contained 34-2-12 (D and H). (A-D) Lysis from cells expressing wild-type  $D^d$  (R8.15.28,  $D^d$  glu); (E-H) lysis from cells expressing the  $\alpha 3$  mutant  $D^d$  (R8.15.29,  $D^d$  lys). The antibodies included in the CTL assay were 34-2-12 (O), 53-6.7 anti-Lyt-2 ( $\square$ ), and a control without antibody ( $\Delta$ ). Additional control antibodies included in the assay were 30-5-7 and 53-7.3, both of which gave the same results as the control without antibody. Unstimulated controls gave no lysis on either of the target cell lines. Spontaneous release from R8.15.28 was 9.8% and from R8.15.29 was 6.4%.

$H-2D^d$ -specific antibody 34-5-8, (Fig. 6). This indicates that qualitative and not quantitative differences account for the functional differences described below.

We therefore examined whether secondary anti- $D^d$  CTL, generated in the presence of antibody to the  $\alpha 3$  domain, could kill cells transfected with the mutant  $D^d$  gene and whether such CTL were Lyt-2 independent. As shown in Fig. 7, primary anti- $D^d$  CTL did not kill cells expressing the mutant  $H-2D^d$  gene, R8.15.29 (Fig. 7 E). Furthermore, the lysis of the cells expressing wild-type  $H-2D^d$  was inhibited by antibody to the  $\alpha 3$  domain of  $D^d$  (34-2-12) and by antibody to Lyt-2 (Fig. 7 A). In contrast, secondary anti- $D^d$  CTL generated in the presence of 34-2-12 in vivo and in vitro killed cells expressing the mutant or the wild-type  $H-2D^d$  gene equally well and this lysis was not inhibited by 34-2-12 and only weakly inhibited by antibody to Lyt-2 (Figs. 7, D and H). To generate this Lyt-2-independent CTL population it was necessary to include the antibody in vitro since in vivo treatment alone was insufficient (Figs. 7, C and G). The observation that cells expressing the mutant  $H-2D^d$  gene were killed by Lyt-2-independent CTL generated in a secondary response, but were not killed by Lyt-2-dependent primary CTL, suggested that the effect of the mutation in the  $H-2D^d$  gene was destruction of the determinant recognized by Lyt-2. Consistent with this interpretation, lysis of R8.15.28 by a subpopulation of secondary CTL generated in the absence of antibody, was not inhibited

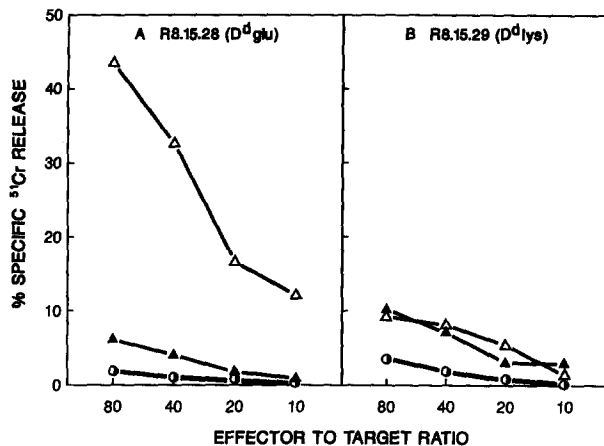


FIGURE 8. The ability of cells transfected with D<sup>d</sup> glu or D<sup>d</sup> lys to stimulate a primary in vitro response. (C3H.OL × BALB.K)F<sub>1</sub> spleen cells were stimulated in vitro with either L.D<sup>d</sup> glu (Δ) or L.D<sup>d</sup> lys (○) and the resulting CTL populations were assayed on R8.15.28 D<sup>d</sup> glu (A) and R8.15.29 D<sup>d</sup> lys (B) target cells in the presence (▲, ●) or absence (Δ, ○) of antibody to Lyt-2. Spontaneous release from R8.15.28 was 6.3% and from R8.15.29 was 5.4%.

by antibody to 34-2-12 or anti-Lyt-2 (Fig. 7 B) and it is this subpopulation of CTL that is capable of lysing the mutant R8.15.29 (Fig. 7 F).

*Cells Expressing a Mutation in the  $\alpha 3$  Domain Fail to Elicit a Primary In Vitro CTL Response.* The data presented thus far demonstrate that the D<sup>d</sup> lys mutant is not recognized by primary CTL generated against the wild-type D<sup>d</sup> glu molecule. However, the D<sup>d</sup> lys molecule is recognized by anti-D<sup>d</sup> CTL generated in secondary responses to wild-type D<sup>d</sup>, especially when they are generated in the presence of mAb to the  $\alpha 3$  domain. We therefore wanted to determine whether a CTL response could be generated using the D<sup>d</sup> lys mutant as the stimulating antigen. If the mutation in the  $\alpha 3$  domain resulted in secondary conformational effects on the  $\alpha 1/\alpha 2$  domains that abrogated recognition by primary CTL, then the mutant D<sup>d</sup> lys molecule should be capable of eliciting a CTL response specific for D<sup>d</sup> lys. If, however, the mutation prevents a necessary interaction between Lyt-2 and the  $\alpha 3$  domain for the generation of primary in vitro CTL, then the D<sup>d</sup> lys antigen might not stimulate a primary response in vitro.

To test whether D<sup>d</sup> lys can stimulate a primary response, naive (C3H.OL × BALB.K)F<sub>1</sub> spleen cells were stimulated with irradiated L cells expressing the wild-type (L.D<sup>d</sup> glu) or mutant (L.D<sup>d</sup> lys) D<sup>d</sup> molecule. This response is confined to H-2D<sup>d</sup> since these F<sub>1</sub> responder spleen cells express antigens encoded by both the C3H background and the H-2<sup>k</sup> haplotype. Thus the only determinants recognized by the F<sub>1</sub> responders on these L cell transfectants should be located on the D<sup>d</sup> lys or D<sup>d</sup> glu molecule respectively. When analyzed by FMF, the L.D<sup>d</sup> glu and L.D<sup>d</sup> lys transfectants expressed equivalent amounts of D<sup>d</sup> molecule as measured by their ability to bind the  $\alpha 1/\alpha 2$ -reactive mAb 34-5-8 (data not shown). The F<sub>1</sub> anti-L.D<sup>d</sup> effectors were tested on <sup>51</sup>Cr-labeled R8.15 transfectants, since these cells serve as better targets than the L cell transfectants. As shown in Fig. 8 A, the wild-type D<sup>d</sup> glu molecule elicited a strong CTL response which was almost completely inhibited by antibody to Lyt-2. Consistent with earlier findings (Fig. 7 and reference 14), these anti D<sup>d</sup>-glu effectors showed only weak cytotoxicity on mutant D<sup>d</sup>-lys antigens and this lysis was not inhibited by anti-Lyt-2 (Fig. 8 B). In contrast, cells expressing the mutant D<sup>d</sup> lys molecules failed to stimulate a primary CTL response when assayed

on cells expressing either the D<sup>d</sup> lys or the D<sup>d</sup> glu molecule (Fig. 8). This observation provides additional evidence that the  $\alpha 3$  domain is involved in Lyt-2 recognition in primary CTL responses.

### Discussion

Characterization of the TCR on subclasses of T cells has led to the observation that the same V $\alpha$  and V $\beta$  chains can be used in receptors restricted by or specific for class I or class II MHC products (25-29). Since the distinction of MHC class by T cells cannot be explained by the usage of nonoverlapping TCR  $\alpha$  and  $\beta$  gene pools, an additional feature of this interaction must provide the basis for the differential recognition. The T cell accessory molecules Lyt-2 (CD8) and L3T4 (CD4) have been implicated as playing a key role in distinguishing class I from class II molecules. A stringent correlation exists between the T cell subset phenotype and the class of MHC gene products recognized by the TCR. The Lyt-2<sup>+</sup>/CD8<sup>+</sup> T cells interact with targets expressing class I MHC antigens and the L3T4<sup>+</sup>/CD4<sup>+</sup> T cells interact with targets expressing class II MHC antigens (11, 30-32).

The functional involvement of the Lyt-2 surface antigen in T cell recognition of class I was first recognized by the demonstration that antibody to Lyt-2 blocks CTL activity (6, 7). It was later proposed that the Lyt-2 receptor interacts with monomorphic determinants on the MHC class I molecules and thus facilitates low-affinity interactions between the TCR and the class I molecule (11, 12). Several reports provide evidence that L3T4/CD4 can enhance T cell responsiveness especially when antigen is limiting and suggest that this is mediated, at least in part, by binding L3T4/CD4 to the class II molecule (33-37) as proposed earlier (11). Thus, both Lyt-2 and L3T4 are thought to perform an adhesive function, facilitating the TCR-MHC interaction. However, it has become increasingly evident that these accessory molecules are involved with additional functions required for the regulation of T cell activation that are independent of MHC antigens (38-43) and that they may not always be essential for T cell function (44). There is also recent evidence that activation results when CD4 or CD8 associates with the appropriate TCR on the cell surface (45-48). These studies also suggest that this is accomplished by the TCR and accessory molecule binding to the same MHC molecule. Therefore, these accessory molecules may facilitate T cell function through a multistep process, part of which involves recognition of a monomorphic determinant on the MHC molecule.

Since T cells that express the Lyt-2 phenotype are associated with class I recognition and antibodies to Lyt-2 can interfere with this recognition it is plausible that the Lyt-2 molecule on the T cell interacts with a monomorphic determinant on the class I molecule on the target cell, thus providing an MHC class I-specific recognition step. Here we present evidence that Lyt-2 recognizes a monomorphic determinant in the  $\alpha 3$  domain of the class I molecule and that this is a distinct site from the one recognized by the TCR. Furthermore, our data suggest that mAb to class I can be used to specifically block either Lyt-2 or TCR recognition. These conclusions are based on three lines of evidence. First, mAbs to the  $\alpha 1/\alpha 2$  and  $\alpha 3$  class I domains show synergism in their ability to enhance L<sup>d</sup>- or D<sup>d</sup>-disparate skin grafts. Second, a correlation was found between the ability of mAb to  $\alpha 3$  and Lyt-2 to block in vitro CTL responses. Furthermore, mAb to  $\alpha 3$  were used to skew the response

such that it was resistant to inhibition by mAb to  $\alpha 3$  and correspondingly less inhibitable by antibody to Lyt-2. The third and most direct evidence supporting the conclusion that Lyt-2 recognizes an  $\alpha 3$  determinant is based on the experiments using a cell line expressing a mutant D<sup>d</sup> (R8.15 D<sup>d</sup> lys) that contains a single amino acid substitution of glu to lys at residue 227 in the  $\alpha 3$  domain. It is noteworthy that position 227 of class I molecules is highly conserved, when comparing available sequences, not only in mice but in rat and human as well (compiled in reference 49). Cells expressing the mutant R8.15 D<sup>d</sup> lys molecule were not lysed by primary CTL generated against wild-type D<sup>d</sup> molecules (13, 14; Fig. 7 E), and the weak lysis of the mutant cell line observed in secondary responses to D<sup>d</sup> (13, Fig. 7 F) was significantly less inhibitable by mAb to  $\alpha 3$  or Lyt-2 when compared with lysis on control R8.15 D<sup>d</sup> glu targets. However, when mAb to  $\alpha 3$  was used both in vivo and in vitro, equal lysis of mutant and wild-type targets by these CTL was observed and this lysis was not inhibited by antibody to  $\alpha 3$  and was only slightly inhibited by antibody to Lyt-2. Furthermore, cells expressing the mutant D<sup>d</sup> lys molecule failed to stimulate a primary CTL response. Therefore, these data suggest that the D<sup>d</sup> lys mutant has lost its ability to be recognized by Lyt-2.

This conclusion would obviously not be justified if secondary effects resulting from this mutation at position 227 influence the  $\alpha 1/\alpha 2$  TCR binding site. We feel this possibility is highly unlikely for the following reasons. First, the binding of mAbs to determinants in the  $\alpha 1$  and/or  $\alpha 2$  domains of the wild-type and mutant D<sup>d</sup> molecules is indistinguishable as measured by fluorometry (14, Fig. 6). Second, T cells whose recognition is Lyt-2-independent, such as the hybridoma 3DT.52.5.8 (14) or the bulk CTL culture shown in Fig. 7, give equal responses on mutant and wild-type targets. Third, based on other structural comparisons, the distance separating residue 227 from the peptide binding site makes the possibility of a secondary interaction unlikely. The recent three-dimensional structure of HLA-A2 revealed that residue 227 is on the surface, but a considerable distance away from the peptide binding site formed by the  $\alpha 1$  and  $\alpha 2$  domains (Dr. D. Wiley, personal communication). The fourth and most direct evidence that the D<sup>d</sup> lys mutation does not alter TCR recognition of the  $\alpha 1/\alpha 2$  binding site is that cells expressing this mutation do not elicit a primary CTL response (Fig. 8). If secondary conformational effects of the mutation were responsible for the observed failure of primary CTL to kill cells expressing the mutant D<sup>d</sup> molecule then cells expressing this mutant molecule should stimulate a primary CTL response specific for the D<sup>d</sup> lys and not the D<sup>d</sup> glu molecule. As shown in Fig. 8, cells expressing the mutant D<sup>d</sup> lys molecule, in contrast to cells expressing the D<sup>d</sup> glu molecule, failed to elicit a primary response. This result suggested that the D<sup>d</sup> lys mutation affected a recognition site of primary CTL that is distinct from the  $\alpha 1/\alpha 2$  site on class I molecules recognized by the TCR. In total, these data strongly support the hypothesis that residue 227 is an integral part of the Lyt-2 binding site on class I molecules.

The results reported here raise several interesting questions concerning in vivo and in vitro recognition of class I molecules. For example, what is the nature of the synergism that results when a combination of antibodies is used to prolong class I-disparate graft survival since either antibody alone has a minimal effect? The results of in vivo antibody blocking studies are obviously more complex since antibodies to the  $\alpha 1/\alpha 2$  domain (30-5-7 for L<sup>d</sup>) are clearly more proficient at abrogating the

in vitro response than the in vivo response. This disparity is likely due to accessibility and time constraint differences between the in vitro and in vivo assays. Perhaps the in vivo situation allows enough time for CTL clones to be generated that displace the antibody or recognize the class I molecule  $\alpha 1/\alpha 2$  domains in the presence of bound antibody. Consistent with this conclusion are the marked differences seen in the ability of several different antibodies to block the in vitro response (50). Therefore, the relatively poor in vivo blocking of antibodies to  $\alpha 1/\alpha 2$ , such as 30-5-7, could be explained by the generation of CTL clones capable of class I recognition in the presence of antibody. Then the synergism of antibody-induced graft enhancement could be explained if these aforementioned CTLs are largely Lyt-2 dependent and thus inhibitable with antibody to the  $\alpha 3$  domain. Another question raised by these studies is whether the Lyt-2<sup>+</sup> T helper cell or the Lyt-2<sup>+</sup> CTL (or both) is blocked in vivo since both cell types appear to be involved in the rejection of skin allografts differing at a single class I locus (51). At the effector cell level, although there was a good correlation between inhibition by antibodies to  $\alpha 3$  and Lyt-2, more inhibition was consistently seen with antibodies to Lyt-2 than with antibodies to  $\alpha 3$ . This suggests that a function in addition to recognition is provided by the Lyt-2/ $\alpha 3$  interaction, such as signal transduction or TCR modulation (45, 46). This interpretation could also explain the partial inhibition of the lysis of cells expressing the mutant D<sup>d</sup> by antibodies to Lyt-2 (Fig. 7).

In conclusion, the results reported here demonstrate that mAbs to class I molecules can be used to selectively block distinct T cell recognition signals. Specifically, mAbs to determinants in the  $\alpha 1/\alpha 2$  domain block TCR recognition, whereas mAbs to determinants in the  $\alpha 3$  domain block Lyt-2 recognition. This observation thus provides a method whereby specific sites on the ligand or class I molecule can be blocked to prevent either Lyt-2 or TCR recognition. For in vitro assays this observation can be used to define the precise cellular and molecular interactions involved in allogeneic, and self-restricted hapten-specific or virus-specific T cell responses. For in vivo responses, our findings suggest that mAb to class I can be used to specifically abrogate recognition of foreign tissue allografts. This blocking of in vivo recognition is in contrast with most of the previous reports of allograft enhancement by antibodies because it is mediated by antibodies to class I and not class II molecules. The extent of prolongation of graft survival that we observed is also greater than that obtained with antibodies to class II molecules. Furthermore, in the system reported here the antibodies used for blocking are exclusively specific for antigens of the allograft and not the host. Thus, this approach is an exquisitely specific way to abolish recognition of tissue transplants.

### Summary

The involvement of the different domains of the MHC class I molecule in CTL recognition was investigated. mAbs specific for the  $\alpha 1/\alpha 2$  domains of H-2L<sup>d</sup> interfered with both the primary and secondary generation and effector function of in vitro L<sup>d</sup>-specific CTL. mAbs specific for the  $\alpha 3$  domain of H-2L<sup>d</sup> interfered with the generation and function of primary in vitro L<sup>d</sup>-specific CTL; however, there was no effect on the in vitro generation of secondary CTL and only partial inhibition of their function. In vivo treatment with graft-specific antibodies to both the  $\alpha 3$  domain and the  $\alpha 1/\alpha 2$  domains together resulted in a dramatic enhancement of L<sup>d</sup>-

or D<sup>d</sup>-disparate skin grafts, whereas the individual mAbs showed minimal effects. This suggested that the class I  $\alpha 3$  domain is recognized by alloreactive CTL. Several approaches were undertaken to examine whether recognition of the  $\alpha 3$  domain determinants is mediated by the Lyt-2 molecule. When mAbs specific for the  $\alpha 3$  domain of either H-2L<sup>d</sup> or H-2D<sup>d</sup> were used in vivo and in vitro, the resulting CTL population was not inhibited by antibody to the  $\alpha 3$  domain and was only partially inhibited by antibody to Lyt-2. We therefore observed a correlation between the effects of antibody to the class I  $\alpha 3$  domain of the target molecule and antibody to the Lyt-2 molecule on the CTL. To further test the relationship between CTL recognition of the  $\alpha 3$  domain and the involvement of Lyt-2, we used a cell expressing a mutation in the  $\alpha 3$  domain of the D<sup>d</sup> molecule. The mutation resulted in a single amino acid substitution of glu to lys at residue 227 of the  $\alpha 3$  domain. Consistent with an earlier report, cells expressing the mutant D<sup>d</sup> lys molecule were not lysed by CTL from a primary stimulation against the wild-type D<sup>d</sup> glu molecule. However, this same cell line was killed by the Lyt-2-independent secondary D<sup>d</sup>-specific CTL generated in the presence of antibody to the  $\alpha 3$  domain in vivo and in vitro. Furthermore, cells expressing the mutant D<sup>d</sup> lys molecule failed to stimulate a primary response. In conclusion, several independent lines of evidence indicate that residues in the  $\alpha 3$  domain of the class I molecule are involved in recognition by the Lyt-2 molecule, and that Lyt-2-mediated recognition can be specifically blocked using mAb to determinants in the  $\alpha 3$  domain.

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### References

1. Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger and D. C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.)* 329:506.
2. Bjorkman, P. J., M.A. Saper, B. Samraoui, W. S. Bennett, J. L. Strominger, and D. C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature (Lond.)* 329:512.
3. Ozato, K., G. A. Evans, B. Skykind, D. H. Margulies, and J. G. Seidman. 1983. Hybrid H-2 histocompatibility gene products assign domains recognized by alloreactive T cells. *Proc. Natl. Acad. Sci. USA* 80:2040.
4. Reiss, C. S., G. A. Evans, D. H. Margulies, J. G. Seidman, and S. J. Burakoff. 1983. Allospecific and virus-specific cytolytic T lymphocytes are restricted to the N or C1 domain of H-2 antigens expressed on L cells after DNA-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* 80:2709.
5. Allen, H., D. Wraith, P. Pala, B. Askonas, and R. A. Flavell. 1984. Domain interactions of H-2 class I antigens alter cytotoxic T-cell recognition sites. *Nature (Lond.)* 309:279.
6. Shinohara, N., and D. H. Sachs. 1979. Mouse alloantibodies capable of blocking cytotoxic T-cell function. I. Relationship between the antigen reactive with blocking antibodies and the Lyt-2 locus. *J. Exp. Med.* 150:432.
7. Nakayama, E., H. Shiku, E. Stockert, H. F. Oettgen, and L. J. Old. 1979. Cytotoxic

- T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. *Proc. Natl. Acad. Sci. USA.* 76:1977.
8. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK 1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.* 131:2445.
  9. Dembic, Z., W. Haas, R. Zamoyska, J. Parnes, M. Steinmetz, and H. von Boehmer. 1987. Transfection of the CD8 gene enhances T-cell recognition. *Nature (Lond.)* 326:510.
  10. Gabert, J., C. Langlet, R. Zamoyska, J. R. Parnes, A.-M. Schmitt-Verhulst, and B. Malissen. 1987. Reconstitution of MHC class I specificity by transfer of the T cell receptor and Lyt-2 genes. *Cell.* 50:545.
  11. Swain, S. L. 1983. T cell subsets and the recognition of MHC class. *Immunol. Rev.* 74:129.
  12. MacDonald, H. R., A. L. Glasebrook, C. Bron, A. Kelso, and J.-C. Cerottini. 1982. Clonal heterogeneity in the functional requirement for Lyt-2/3 molecules on cytolytic T lymphocytes (CTL): possible implications for the affinity of CTL antigen receptors. *Immunol. Rev.* 68:89.
  13. Potter, T. A., M. A. Palladino, D. B. Wilson, and T. V. Rajan. 1983. Epitopes on H-2D<sup>d</sup> somatic cell mutants recognized by cytotoxic T cells. *J. Exp. Med.* 158:1061.
  14. Potter, T. A., J. A. Bluestone, and T. V. Rajan. 1987. A single amino acid substitution in the  $\alpha$ 3 domain of an H-2 class I molecule abrogates reactivity with CTL. *J. Exp. Med.* 166:956.
  15. Ozato, K., T. H. Hansen, and D. H. Sachs. 1980. Monoclonal antibodies to mouse MHC antigens. II. Antibodies to the H-2L<sup>d</sup> antigen, the products of a third polymorphic locus of the mouse major histocompatibility complex. *J. Immunol.* 125:2473.
  16. Evans, G. A., D. A. Margulies, B. Shykind, J. G. Seidman, and K. Ozato. 1982. Exon shuffling: mapping polymorphic determinants on hybrid mouse transplantation antigens. *Nature (Lond.)* 300:755.
  17. Ozato, K., N. M. Mayer, and D. H. Sachs. 1982. Monoclonal antibodies to mouse MHC antigens. IV. A series of hybridoma clones producing anti-H-2<sup>d</sup> antibodies and an examination of expression of H-2<sup>d</sup> antigens on the surface of these cells. *Transplantation (Baltimore)* 34:113.
  18. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.
  19. Ledbetter, J. A., R. V. Rouse, H. S. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J. Exp. Med.* 152:280.
  20. Hansen, T. H., S. L. Koprak, E.-M. Wormstall, B. J. Olson, and R. D. Jackson. 1985. Long-term passive enhancement of allogeneic skin grafts with monoclonal antibodies. *J. Immunogenet. (Oxf.)* 12:167.
  21. Zeff, R. A., J. Gopas, E. Steinhauer, T. V. Rajan, and S. G. Nathenson. 1986. Analysis of somatic cell H-2 variants to define the structural requirements for class I antigen expression. *J. Immunol.* 137:897.
  22. Rubocki, R. J., T. H. Hansen, and D. R. Lee. 1986. Molecular studies of murine mutant BALB/c-H-2<sup>dm2</sup> define a deletion of several class I genes including the entire H-2L<sup>d</sup> gene. *Proc. Natl. Acad. Sci. USA.* 83:9606.
  23. McKenzie, I. F. C., and G. D. Snell. 1973. Comparative immunogenicity and enhancement of individual H-2K and H-2D specificities of the murine histocompatibility-2 complex. *J. Exp. Med.* 138:259.
  24. Lems, S. P. M., C. W. M. Jacobs, P. J. A. Capel, and R. A. P. Koene. 1987. Effects

- of monoclonal anti-H-2L<sup>d</sup> and anti-H-2D<sup>d</sup> alloantibodies in the immunological enhancement of skin grafts and neonatal heart grafts in the mouse. *J. Immunol.* 138:2082.
25. Rupp, F., H. Acha-Orbea, H. Hengartner, R. Zinkernagel, and R. Joho. 1985. Identical V $\beta$  T-cell receptor genes used in alloreactive cytotoxic and antigen plus I-A specific helper T cells. *Nature (Lond.)* 315:425.
  26. Barth, R. K., B. S. Kim, N. C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershengfeld, C. Okada, D. Hansburg, I. L. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed V $\beta$  gene segments. *Nature (Lond.)* 316:51.
  27. Garman, R. D., J.-L. Ko, C. D. Vulpe, and D. H. Raulet. 1986. T-cell receptor variable region gene usage in T-cell populations. *Proc. Natl. Acad. Sci. USA.* 83:3987.
  28. Blackman, M., M. Yague, R. Kubo, D. Gay, C. Coleclough, E. Palmer, J. Kappler, and P. Marrack. 1986. The T cell repertoire may be biased in favor of MHC recognition. *Cell.* 47:349.
  29. Kappler, J. W., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T cell receptor V $\beta$  segment that imparts reactivity to a class II major histocompatibility complex product. *Cell.* 49:263.
  30. Swain, S. L. 1981. Significance of Lyt phenotypes: Lyt-2 antibodies block activities of T cells that recognize class I major histocompatibility complex antigens regardless of their function. *Proc. Natl. Acad. Sci. USA.* 78:7101.
  31. Krensky, A. M., C. S. Reiss, J. W. Mier, J. L. Strominger, and S. J. Burakoff. 1982. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4<sup>+</sup>. *Proc. Natl. Acad. Sci. USA.* 79:2365.
  32. Biddison, W. E., P. E. Rao, M. A. Talle, G. Goldstein, and S. Shaw. 1982. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens. Evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J. Exp. Med.* 156:1065.
  33. Sleckman, B. P., A. Peterson, W. K. Jones, J. A. Foran, J. L. Greenstein, B. Seed, and S. J. Burakoff. 1987. Expression and function of CD4 in a murine T-cell hybridoma. *Nature (Lond.)* 328:351.
  34. Gay, D., P. Maddon, R. Sekaly, M. A. Talle, M. Godfrey, E. Long, G. Goldstein, L. Chess, R. Axel, J. Kappler, and P. Marrack. 1987. Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA-DR antigen. *Nature (Lond.)* 328:626.
  35. Greenstein, J. L., J. Kappler, P. Marrack, and S. J. Burakoff. 1984. The role of L3T4 in recognition of Ia by a cytotoxic, H-2D<sup>d</sup>-specific T cell hybridoma. *J. Exp. Med.* 159:1213.
  36. Gay, D., C. Coeshott, W. Golde, J. Kappler, and P. Marrack. 1986. The major histocompatibility complex-restricted antigen receptor on T cells. IX. Role of accessory molecules in recognition of antigen plus isolated IA. *J. Immunol.* 136:2026.
  37. Doyle, C., and J. L. Strominger. 1987. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature (Lond.)* 330:256.
  38. Hunig, T. 1984. Monoclonal anti Lyt-2.2 antibody blocks lectin-dependent cellular cytotoxicity of H-2-negative target cells. *J. Exp. Med.* 159:551.
  39. Bekoff, M., T. Kakiuchi, and H. M. Grey. 1985. Accessory cell function in the Con A response: role of Ia-positive and Ia-negative accessory cells. *J. Immunol.* 134:1337.
  40. Bank, I., and L. Chess. 1985. Perturbation of the T4 molecule transmits a negative signal to T cells. *J. Exp. Med.* 162:1294.
  41. Fleischer, B., H. Schrezenmeier, and H. Wagner. 1986. Function of the CD4 and CD8 molecules on human cytotoxic T lymphocytes: regulation of T cell triggering. *J. Immunol.* 136:1625.



42. Van Seventer, G. A., R. A. W. Van Lier, H. Spits, P. Ivanyi, and C. J. M. Melief. 1986. Evidence for a regulatory role of the T8 (CD8) antigen in antigen-specific and anti-T3-(CD3)-induced lytic activity of allospecific cytotoxic T lymphocyte clones. *Eur. J. Immunol.* 16:1363.
43. Blue, M.-L., D. A. Hafler, J. F. Daley, H. Levine, K. A. Craig, J. B. Breitmeyer, and S. F. Schlossman. 1988. Regulation of T cell clone function via CD4 and CD8 molecules. Anti-CD4 can mediate two distinct inhibitory activities. *J. Immunol.* 140:376.
44. Yui, K., Y. Hashimoto, S. Wadsworth, and M. I. Greene. 1987. Characterization of Lyt-2<sup>-</sup>, L3T4<sup>-</sup> class I-specific cytolytic clones in C3H-gld/gld mice. Implications for functions of accessory molecules and programmed development. *J. Exp. Med.* 166:1026.
45. Emmrich, F., U. Strittmatter, and K. Eichmann. 1986. Synergism in the activation of human CD8 T cells by cross-linking the T-cell receptor complex with the CD8 differentiation antigen. *Proc. Natl. Acad. Sci. USA.* 83:8298.
46. Takada, S., and E. G. Engleman. 1987. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. *J. Immunol.* 139:3231.
47. Saizawa, K., J. Rojo, and C. A. Janeway, Jr. 1987. Evidence for a physical association of CD4 and the CD3:α:β T-cell receptor. *Nature (Lond.)* 328:260.
48. Kupfer, A., S. J. Singer, C. A. Janeway, Jr., and S. L. Swain. 1987. Co-clustering of CD4 (L3T4) molecule with the T-cell receptor is induced by specific direct interaction of helper T cells and antigen-presenting cells. *Proc. Natl. Acad. Sci. USA.* 84:5888.
49. Klein, J., and F. Figueroa. 1986. The evolution of class I MHC genes. *Immunol. Today.* 7:41.
50. Forman, J. 1987. Determinants on major histocompatibility complex class I molecules recognized by cytotoxic T lymphocytes. *Adv. Immunol.* 41:135.
51. Rosenberg, A. S., T. Mizuochi, and A. Singer. 1986. Analysis of T-cell subsets in rejection of K<sup>b</sup> mutant skin allografts differing at class I MHC. *Nature (Lond.)* 322:829.