

MONOCLONAL ANTI-LYT-2.2 ANTIBODY BLOCKS LECTIN-
DEPENDENT CELLULAR CYTOTOXICITY OF
H-2-NEGATIVE TARGET CELLS

BY THOMAS HÜNIG

*From the Institut für Virologie und Immunbiologie, D-8700 Würzburg, Federal Republic of
Germany*

During the past years, a number of T cell antigens defined by conventional serology and monoclonal antibodies have become the basis for the distinction of T cell subsets, especially in the human, murine, and rat systems (reviewed in reference 1). Some of these markers have been directly correlated with T cell functions, either because they are only expressed on a given functional subset or because antibodies to them block or stimulate certain T cell reactions. A well studied example is the murine Lyt-2 (human OKT8/Leu-2) antigen. Originally thought to be a marker for cytotoxic (and suppressor) T cells (2), more recent data suggest an association of this antigen with T cell recognition of class I major histocompatibility complex (MHC)¹ antigens rather than with cytolytic function (3). Thus, Class I-specific helper T cells were found to be Lyt-2⁺ as well (4), and conversely, class II specific cytolytic T lymphocytes (CTL) with little or no Lyt-2 antigen were described (5). In the human, an analogous antigen is known to be associated with the recognition of class II MHC antigens (OKT4/Leu-3) (6, 7), and its likely murine counterpart (L3T4) has been described recently (8). The strongest evidence for an involvement of the molecules bearing these antigens in the recognition of MHC restriction elements is the capacity of anti-Lyt-2 (3) and anti-L3T4 (8) antibodies (and their counterparts in the human system [9]) to block the activities of cytotoxic and helper T cells that recognize class I and class II antigens, respectively. Accordingly, it has been proposed that the T cell structures defined by these antibodies interact with monomorphic determinants on MHC molecules, thereby providing an MHC class-specific recognition step in addition to the T cell receptors' interaction with its target antigen (3).

Recently, evidence has been presented suggesting that the mechanism by which lectins polyclonally activate T cells (10) and mediate nonspecific (CTL)-lysis (11) (lectin-dependent cellular cytotoxicity, LDCC) is similar or identical to that operating in antigen-specific systems. Thus, it has been postulated that T cells recognize lectin-modified MHC antigens on "accessory" and target cells. The finding that LDCC (12) and lectin-induced reactivity to interleukin 2 (IL-2)

This work was supported by Sonderforschungsbereich 105, Würzburg.

¹ *Abbreviations used in this paper:* CTL, cytotoxic T lymphocytes; Con A, concanavalin A; IL-2, interleukin 2; LDCC, lectin-dependent cellular cytotoxicity; LFA, lymphocyte-function associated antigen; MHC, major histocompatibility complex; NK, natural killer cells.

(13) can be blocked with monoclonal anti-Lyt-2 has been interpreted as a key result supporting this hypothesis (14). On the other hand, H-2-negative cells can serve as targets in LDCC (15) and as accessory cells in polyclonal T cell activation.² In the present report, the hypothesis that anti-Lyt-2 blocks at the level of MHC class recognition was tested by examining its capacity to inhibit LDCC of H-2-positive and H-2-negative target cells.

Materials and Methods

Mice. Balb.K (H-2^k, Lyt-2.2), CBA/J (H-2^k, Lyt-2.1), and BALB/c (H-2^d, Lyt-2.2) mice were bred in our colony and used at 2–6 months of age.

Generation of Cytotoxic Effector Cells. Spleen cells were cultured in RPMI 1640 supplemented as given in reference 16. They were either stimulated for 3 d with concanavalin A (Con A) (2 µg/ml) at 2 × 10⁶/ml in 2-ml cultures seeded into 24-well tissue culture plates (Costar, Data Packaging, Cambridge, MA), or for 5 d with irradiated (2,000 rads from a ⁶⁰Co source) allogeneic spleen cells as described (16). Con A-induced CTL were incubated with 50 mM α-methyl-mannoside for 15 min, followed by two washes, before the CTL assay.

Target Cells. The C58/J (H-2^k, Lyt-2.1) thymoma R1.1 and the β₂-microglobulin (and therefore H-2^k) negative variant R1E/TL8x.1 (for short, R1.E), which were derived and characterized by Hyman and Stallings (17), were kindly provided by Dr. K. Fischer-Lindahl (Basel Institute for Immunology). They were labeled with 100 µCi ⁵¹Cr-Na₂CrO₄ in 0.5 ml BSS/BSA in the presence or in the absence of 5 µg Con A, followed by two washes.

Antibodies. The hybridoma 19/1782c producing anti-Lyt-2.2 antibody of the IgG2a subclass was kindly provided by Dr. G. Hämmerling (DKFZ, Heidelberg, FRG). IgG was prepared from ascitic fluid by ammonium sulfate precipitation and fractionation on an Affigel blue column (Biorad Laboratories GmbH, München, FRG). As judged by protein staining of SDS-PAGE, the recovered material contained almost exclusively IgG. Anti LFA-1 (18), the kind gift of Dr. M. Pierres (Centre D'Immunologie de Marseille-Luminy), was used in the form of culture supernatant (hybridoma clone H 35-89.9 rat γ_{2b}).

CTL Assay (16). The cytotoxic effector cells were preincubated with the monoclonal antibodies in a total volume of 0.5 ml supplemented (16) RPMI for 30 min at 4°C in 120 mm × 7.5 mm polystyrene tubes. 4 × 10⁴ target cells were added in another 0.5 ml of medium, and the mixture was incubated for 4 h at 37°C in an atmosphere containing 5% CO₂ in air. After mixing and centrifugation at 2,000 rpm for 5 min, 0.5-ml samples of the supernatants were counted. Specific lysis was calculated according to the formula:

$$\% \text{ specific lysis} = 100 \times \frac{\text{experimental release} - \text{spontaneous release}}{\text{detergent release} - \text{spontaneous release}}$$

Results

Blocking of Specific and Lectin-dependent Lympholysis Mediated by Alloreactive CTL with Monoclonal Anti-Lyt-2.2 Antibody. Fig. 1 shows an experiment in which BALB/c anti-Balb.K (H-2^d anti-H-2^k) CTL were tested against the C58/J thymoma R1.1 (H-2^k) and the β₂-microglobulin defective and therefore H-2^k negative variant, R1.E. As expected, only the H-2^k-positive target cells were lysed (Fig. 1A). However, pretreatment with Con A rendered R1.E cells susceptible to lysis as well (Fig. 1C). This is in agreement with results of Bevan and Hyman (15), who first described LDCC of H-2-negative cells. Inclusion of 10 µg/ml purified anti-Lyt-2.2 antibody markedly reduced both the antigen-specific (Fig.

²Hünig, T. 1983. The role of accessory cells in polyclonal T cell activation. III. No requirement for recognition of H-2 antigens on accessory cells. Submitted for publication.

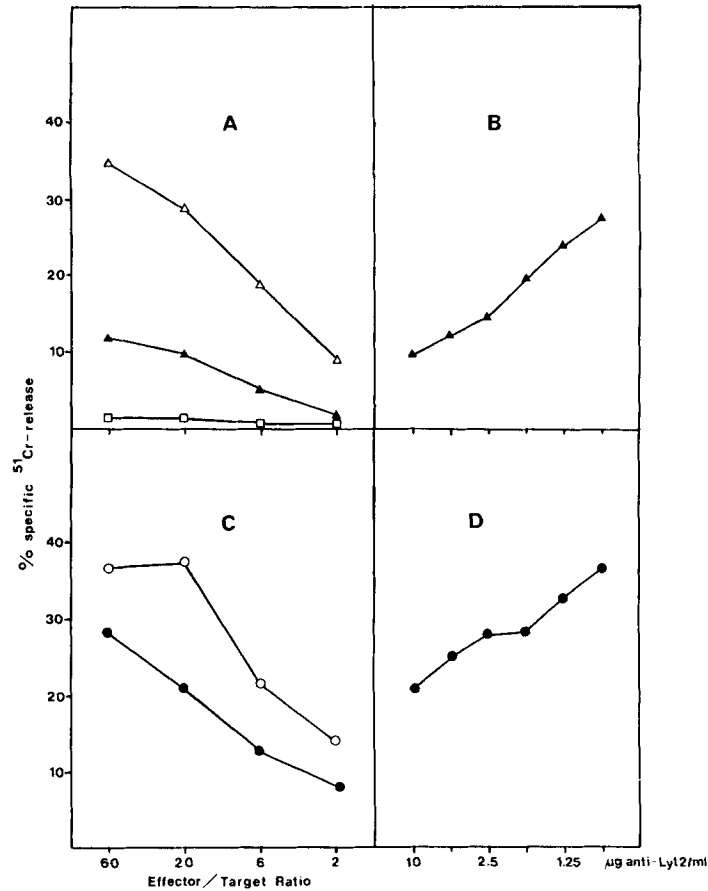


FIGURE 1. Antigen-specific lysis of H-2⁺ and LDCC of H-2⁻ target cells by alloreactive CTL is inhibited by anti-Lyt-2.2. BALB/c (H-2^d) anti-Balb.K (H-2^k) CTL were tested against R 1.1 (H-2^k) (△, ▲), R 1.E (H-2^d) (□), and Con A-pulsed R 1.E (○, ●) target cells in the presence (▲, ●) or in the absence (△, ○) of purified anti-Lyt-2.2. (A, C) Titration of effector cells ±10 µg/ml anti-Lyt-2.2. (B, D) Titration of anti-Lyt-2.2 at a constant effector/target ratio of 20:1. Spontaneous release varied from 14.4 to 25.8%.

1A) lysis of H-2^k positive and LDCC of H-2⁻ target cells (Fig. 1C). Titration of the antibody at a constant effector/target ratio (Fig. 1, B and D) indicated that similar antibody concentrations were required for inhibition in both systems.

Blocking of LDCC Mediated by Polyclonally Induced CTL with Anti-Lyt-2.2 and Anti-LFA-1. In the experiment shown in Fig. 2, Con A-pulsed R 1.1 (Fig. 2, A and B) and R 1.E (Fig. 2, C and D) cells were used as targets for polyclonally (Con A-) induced CTL. LDCC of the H-2⁻ target was less than that of the H-2⁻ positive parent line (note the different scale), but was significant and reproducible. As can be seen, monoclonal anti-Lyt-2.2 inhibited lysis of both targets. In addition, culture supernatants from a hybridoma producing anti-LFA-1 antibody were inhibitory in both systems. Unlike Lyt-2, LFA-1 expression is not correlated with class I or class II MHC recognition (8). Again, similar

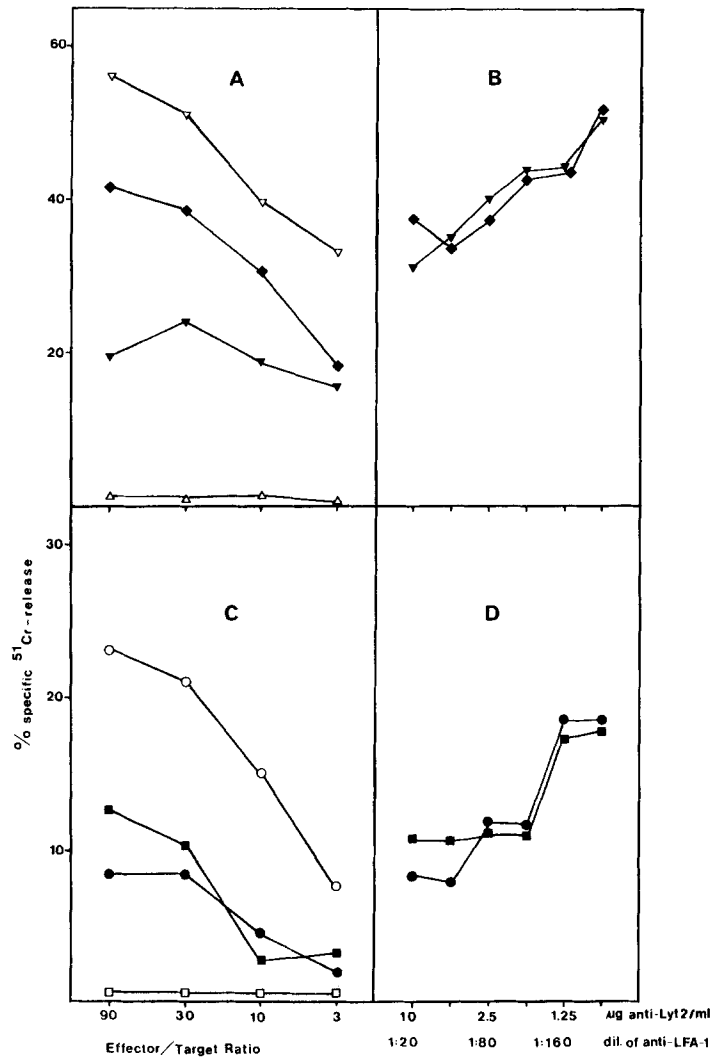


FIGURE 2. Inhibition of LDCC of $H-2^+$ and $H-2^-$ target cells by anti-Lyt-2.2 and anti-LFA-1. Con A-induced CTL from Balb.K mice were tested against R 1.1 (Δ), R 1.E (\square), and Con A-pulsed R 1.1 (∇ , \blacklozenge , \blacktriangledown) and R 1.E (\circ , \bullet , \blacksquare) target cells in the absence of inhibiting antibodies (∇ , \circ), or in the presence of anti-Lyt-2.2 (\blacktriangledown , \bullet), or of anti-LFA-1 (\blacklozenge , \blacksquare). (A, C) Titration of effector cells $\pm 20 \mu\text{g/ml}$ anti-Lyt-2.2 or anti-LFA-1 at 1:20 final. (B, D) Titration of inhibitory antibodies at a constant effector to target ratio of 30:1. Spontaneous release varied from 12.9 to 29.9%.

titration curves were obtained for these antibodies in the blocking of LDCC of $H-2$ -positive and $H-2$ -negative target cells. While the blocking of LDCC of both types of target cells with anti-Lyt-2.2 and anti-LFA-1 was a consistent finding, the relative efficiency with which the two antibodies inhibited was variable from experiment to experiment. The allele specificity of blocking with monoclonal anti-Lyt-2.2 antibody is shown in Table I. Lysis of Con A-pulsed R 1.1 and R 1.E target cells by Balb.K (Lyt-2.2) but not by CBA (Lyt-2.1) Con A-activated

TABLE I
Monoclonal Anti-Lyt-2.2 Inhibits LDCC of H-2⁺ and H-2⁻ Target Cells in an Allele-specific Manner

Effector cells*	Lyt-2 allele	Target cell	Antibody added‡	% specific ⁵¹ Cr release	
Balb.K	2.2	R 1.1	—	0.2	
Balb.K		R 1.1-Con A	—	56.5	
Balb.K		R 1.1-Con A	Anti-Lyt-2.2	34.4	
Balb.K		R 1.1-Con A	Anti-LFA-1	24.8	
Balb.K		R 1.E	—	-0.1	
Balb.K		R 1.E-Con A	—	20.0	
Balb.K		R 1.E-Con A	Anti-Lyt-2.2	10.1	
Balb.K		R 1.E-Con A	Anti-LFA-1	8.1	
CBA		2.1	R 1.1	—	1.3
CBA			R 1.1-Con A	—	51.5
CBA	R 1.1-Con A		Anti-Lyt-2.2	48.3	
CBA	R 1.1-Con A		Anti-LFA-1	22.9	
CBA	R 1.E		—	1.2	
CBA	R 1.E-Con A		—	22.8	
CBA	R 1.E-Con A		Anti-Lyt-2.2	22.4	
CBA	R 1.E-Con A		Anti-LFA-1	10.0	

* Con A-activated spleen cells. Effector/target ratio 60:1.

‡ Anti-Lyt-2.2: 10 µg/ml final; anti-LFA-1: 1:20 dilution of culture supernatant.

CTL was blocked. In contrast, anti-LFA-1, a rat antibody reactive with LFA-1 on the cells of all mouse strains, was inhibitory for effector cells from both strains.

Discussion

The experiments presented here show that monoclonal anti-Lyt-2 antibodies can block LDCC of H-2-negative target cells. The minimal conclusion to be drawn is that in LDCC, blocking with anti-Lyt-2 antibodies cannot be taken as evidence for the recognition of class I H-2 antigens in LDCC. Unless two distinct mechanisms by which anti-Lyt-2 blocks T cell-mediated cytotoxicity are to be postulated, this conclusion can be extrapolated to antigen-specific T cell killing as well.

Basically, two functions have been suggested for the Lyt-2 bearing T-cell surface molecule: strengthening of adhesion between CTL and target cell (12, 20), and recognition of class I MHC antigens (3). Both proposals take into account that inhibition of cytolysis by anti-Lyt-2 antibodies operates at the level of CTL-target cell conjugate formation (21). The finding that antibodies to LFA-1 (18) interfere with the same step in T cell-mediated target cell destruction (20) seems to put Lyt-2 and LFA-1 into the same category. Unlike blocking by anti-LFA-1, however, which affects T cells recognizing H-2 class I and class II antigens, sensitivity to inhibition by anti-Lyt-2 is considered a property of only class I-specific T cells (3). The idea that the Lyt-2-bearing molecule is itself the T cell receptor had to be discarded with the discovery of codominant expression

of both Lyt-2 alleles on the same cell (22). Instead, it has been proposed that Lyt-2 is part of the T cell receptor complex, where it functions as a MHC class-I specific receptor by interacting with monomorphic determinants on class I molecules (3). Exceptions have, however, been found to the rule that class I MHC recognition is associated with Lyt-2 expression (23, 24), and some Lyt-2⁺ CTL are resistant to blocking with anti-Lyt-2 antibodies (25). In view of these findings, the involvement of the Lyt-2 antigen in the adhesion of class I-specific T cells to their targets has more cautiously been interpreted as a "stabilizing" effect that is required only in low affinity interactions of the T cell receptor with class I antigens (25).

According to the data presented here, sensitivity to blocking with anti-Lyt-2 can not be regarded as evidence for the recognition of class I MHC antigens. On the other hand, the predominant *expression* of Lyt-2 on class I-specific T cells remains suggestive of its involvement in the T cell receptor complex. Thus, it could be envisaged that even in LDCC of H-2-negative target cells, participation of the T cell receptor complex is required. If the Lyt-2 antigen is part of that complex, blocking of both LDCC and antigen-specific killing may proceed by a mechanism distinct from interfering with the postulated interaction of the Lyt-2-bearing structure with monomorphic class I determinants.

A result possibly analogous to the one reported here about anti-Lyt-2 was recently published for the monoclonal antibody against L3T4, the presumptive murine analogue to the human T4 antigen, which is found associated with class II recognition (8). This antibody was selected in a screening assay in which LDCC of P815 (i. e. class II negative) target cells by a T cell clone of unknown (but presumably class II restricted) specificity was blocked.

Finally, the blocking of LDCC of H-2-negative target cells with anti-Lyt-2 indicates that CTL, and not natural killer (NK) cells, are the effector cells in this system. This supports the notion that T cell reactivities mediated by lectins need not involve MHC antigens on target (or "accessory") cells (15).²

Summary

The hypothesis that blocking of cytotoxic T lymphocyte (CTL)-mediated cytolysis with anti-Lyt-2 antibodies acts at the level of inhibiting the interaction of the Lyt-2-bearing structure with H-2 class I molecules was tested. In agreement with the findings of others, purified anti-Lyt-2.2 inhibited both antigen-specific lysis and lectin-dependent cellular cytotoxicity (LDCC). LDCC of H-2-positive and H-2-negative target cells was similarly inhibited by this antibody. As expected, this effect was specific for CTL expressing the Lyt-2.2 allele, in contrast to blocking with a rat monoclonal antibody to the murine LFA-1 antigen. The implications of this finding for the function of the Lyt-2 antigen in CTL-target cell interaction are discussed.

I thank Drs. A. Schimpl, E. Wecker, and H.-W. Vohr for helpful discussion, N. Rosenthal for excellent technical assistance, and Ch. Stoppe for the preparation of this manuscript.

Received for publication 26 October 1983.

References

1. Möller, G., editor. 1983. Functional T-cell subsets defined by monoclonal antibodies. *Immunol. Rev.* Vol. 74.
2. Cantor, H., and E. A. Boyse. 1976. Regulation of cellular and humoral immune responses by T-cell subclasses. *Cold Spring Harbor Symp. Quant. Biol.* 41:23.
3. Swain, S. L. 1983. T-cell subsets and the recognition of MHC class. *Immunol. Rev.* 74:129.
4. Swain, S. L., and P. R. Panfili. 1979. Helper cells activated by allogeneic H-2 K or H-2 D differences have an Ly phenotype distinct from those responsive to I differences. *J. Immunol.* 122:383.
5. Swain, S. L., G. Dennert, S. Wormsley, and R. W. Dutton. 1981. The Lyt phenotype of a long term allospecific T-cell line. Both helper and killer activities to Ia are mediated by Ly 1 cells. *Eur. J. Immunol.* 11:175.
6. 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⁺. *Proc. Natl. Acad. Sci. USA.* 79:2365.
7. 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 S. B. antigens. *J. Exp. Med.* 156:1065.
8. Dialynas, D. P., D. B. Wilde, P. Marrack, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch. 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK 1.5: expression of L3T4a by functional T-cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.* 74:29.
9. Reinherz, E. L., S. C. Meuer, and S. F. Schlossman. 1983. The human T-cell receptor: analysis with cytotoxic T-cell clones. *Immunol. Rev.* 74:83.
10. Kimura, A., and B. Ersson. 1981. Activation of T lymphocytes by lectins and carbohydrate-oxidizing reagents viewed as immunological recognition of cell surface modifications in the context of "self" MHC antigens. *Eur. J. Immunol.* 11:475.
11. Berke, G., V. Hu, E. McVey, and W. R. Clark. 1981. T-lymphocyte-mediated cytotoxicity. II. Role of target cell histocompatibility antigens in recognition and lysis. *J. Immunol.* 127:782.
12. Bonavida, B., T. Bradley, J. Fan, J. Hiserodt, R. Effros, and H. Wexler. 1983. Molecular interactions in T-cell mediated cytotoxicity. *Immunol. Rev.* 72:119.
13. Gullberg, M., and E. L. Larsson. 1983. Induction of cytolytic T-lymphocytes is specifically inhibited by anti-Lyt 2 antibodies. *Eur. J. Immunol.* 13:476.
14. Larsson, E. L., M. Gullberg, A. Beretta, and A. Coutinho. 1982. Requirement for the involvement of clonally distributed receptors in the activation of cytotoxic T-lymphocytes. *Immunol. Rev.* 68:69.
15. Bevan, M. J., and R. Hyman. 1977. The ability of H-2⁺ and H-2⁻ cell lines to induce or be lysed by cytotoxic T-cells. *Immunogenetics.* 4:7.
16. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766.
17. Hyman, R., and V. Stallings. 1976. Characterization of a TL⁻ variant of a homozygously TL⁺ mouse lymphoma. *Immunogenetics.* 3:75.
18. Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T. A. Springer. 1981. Monoclonal antibody to a novel lymphocyte function associated antigen (LFA-1): mechanism of blocking T-lymphocyte mediated killing and effects on other T- and B-lymphocyte functions. *J. Immunol.* 127:590.
19. Pierres, M., C. Goridis, and P. Golstein. 1982. Inhibition of murine T-cell mediated

- cytolysis and T-cell proliferation by a rat monoclonal antibody immunoprecipitating two lymphoid cell surface peptides of 94,000 and 180,000 molecular weight. *Eur. J. Immunol.* 12:60.
20. Martz, E., W. Heagy, and S. H. Gromkowski. 1983. The mechanism of T-cell mediated killing: monoclonal antibody analysis of the roles of killer and target-cell membrane proteins. *Immunol. Rev.* 72:73.
 21. Fan, J., A. Ahmed, and B. Bonavida. 1980. Studies on the induction and expression of T-cell mediated immunity. X. Inhibition by Lyt 2.3 antisera of cytotoxic T-lymphocyte mediated antigen-specific and non-specific cytotoxicity: evidence for the blocking of the binding between T-lymphocytes and not the post-binding cytolytic steps. *J. Immunol.* 125:2444.
 22. Ledbetter, J. A., W. E. Seaman, T. T. Tsu, and L. A. Herzenberg. 1981. Lyt-2 and Lyt-3 antigens are on two different polypeptide subunits linked by disulfide bonds. Relationships of subunits to T cell cytolytic activity. *J. Exp. Med.* 153:1503.
 23. Kaufmann, Y., P. Golstein, M. Pierres, T. Springer, and Z. Eshhar. 1982. LFA-1 but not Lyt 2 is associated with killing activity of cytotoxic T lymphocyte hybridomas. *Nature (Lond.)* In press.
 24. Giorgi, J. V., J. A. Zawadski, and N. L. Warner. 1982. Cytotoxic T-lymphocyte lines reactive against murine plasmacytoma antigens: dissociation of cytotoxicity and Lyt 2 expression. *Eur. J. Immunol.* 12:831.
 25. Mac Donald, H. R., A. L. Glasebrook, and J.-C. Cerottini. 1982. Clonal heterogeneity in the functional requirement for Lyt-2/3 molecules on cytolytic T-lymphocytes: analysis by antibody blocking and selective trypsinization. *J. Exp. Med.* 156:1711.