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# THE MOLECULAR BASIS FOR CYTOLYTIC T LYMPHOCYTE FUNCTION: ANALYSIS WITH BLOCKING MONOCLONAL ANTIBODIES

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## INTRODUCTION

Cytolytic T lymphocytes (CTL) were identified during the late 1960's (1,2) and are thought to be important effector cells in immunity to viruses (3) and in allograft rejection (4). Despite considerable effort during the past decade, little has been learned about the biochemical basis for the killing event. Not only do the presumed molecular mediators remain unidentified, but fundamental physiological questions remain unanswered.

The primary target cell site damaged by the CTL has not been identified. While electrolyte fluxes have been observed at the time of programming for lysis (5), the sodium influx or depolarization of membrane potential expected to ensue if membrane "holes" have been created (similar to those produced by complement) have not been documented.

It is often supposed that the primary lesions are in the target cell membrane, and that these lead to colloid osmotic lysis of the sort documented for complement-mediated lysis of erythrocytes. However, a colloid osmotic lytic phenomenon has not been substantiated for viable nucleated cells, such as tumor cells, even in the case of complement (a view also held by Sanderson, 6). Such cells are capable of considerably more complicated responses to damage than are red cells (7,8). Experiments employing macromolecular "osmotic protectants" to estimate the size of the membrane lesions

(?) induced by CTLs are open to alternative interpretations (6; section VI.C in 9).

What, then, have we learned about the mechanism of CTL-mediated killing during the past decade?

1. The CTL is not only antigenically specific, but, once it recognizes a target, remains exquisitely selective in sparing antigenically "innocent" bystander cells (4). This excludes release of a stable, non-specific toxin. A labile and/or specific toxin remains possible (Cf. 10).
2. The CTL remains unharmed during killing, and can kill multiple target cells sequentially in time (11,12).
3. Nevertheless, CTLs can be killed by other CTLs (13,14), although this leaves open the possibility of spatially or temporally limited resistance to their own toxin.
4. The target cell probably plays no active role in its own demise, since target cells whose metabolism has been irreversibly poisoned by glutaraldehyde are killed efficiently (15).
5. Once differentiated, the CTL needs no protein synthesis during killing, probably even to kill repeatedly (16).
6. Some lectins induce CTLs to kill nonspecifically (17) by a mechanism very similar to that employed in specific killing (18). Nevertheless, mere adhesion with a CTL is not sufficient for killing (19), suggesting that the lytic mechanism requires triggering by the antigen receptors. This conclusion is also consistent with the outcome of elegant unidirectional recognition experiments involving two CTL populations (20).
7. Individual CTL cells can vary in killing efficiency. Secondary CTL form conjugates in half the time required by primary CTL (89), and in individual binary conjugates, secondary CTL kill their targets in half the time required by primary CTL (21).
8. Assays have been developed which resolve the killing process into three steps: recognition-adhesion (probably two events but not yet clearly resolved), programming for lysis (the lethal hit), and killer cell-independent lysis (9,22).
9. Completion of programming for lysis can occur within minutes after contact with the target (23,24).
10. The recogniton-adhesion step requires $Mg^{++}$; $Ca^{++}$ is unnecessary and insufficient (25). Programming for lysis is calcium (or strontium) dependent (22,26,27); killer cell independent lysis requires neither divalent cation (9).
11. Cytochalasins inhibit primarily or solely the recognition-adhesion step (28,29), while other pharmacologic inhibitors appear to act both on recognition-adhesion and on programming for lysis (29,30).
12. It seems likely that the primary lesion produced by CTLs

in the target cell is functionally different from that produced by complement, based on studies with mast cell targets (31,32, and Martz, Parker, Gately, and Tsoukas, this volume) and on morphologic observations of lysis (6).

## REASONS FOR LACK OF MOLECULAR UNDERSTANDING OF CTL FUNCTION

Molecular understanding of the mechanism of CTL-mediated killing has been slow to develop. With the exception of Hiserodt's tantalizing but unconfirmed report of a labile antigen-specific lysis produced by CTL-rich T lymphocytes (10), attempts to detect lytic effector molecules in CTL extracts or supernatants have been unrewarding (9,33). No T cell antigen-receptor is well understood in molecular terms at the present time, including the CTL receptor (Cf. 34). Even attempts to isolate target H-2 antigens in a form which will bind specifically to CTL, much less competitively block killing, have been largely unsuccessful (35-39). Finally, it has not been feasible to simplify the target cell, for example, to an erythrocyte (there are no reports of specific CTL-mediated killing of erythrocytes) or a liposome (Cf. 40).

## SEARCH FOR PROTEINS UNIQUE TO CTL

A more general approach to a molecular understanding of CTL-mediated killing is to search for unusual or unique CTL proteins. Kimura (41) reported that Lyt-$2^+$ (but not Lyt-$2^-$) T lymphoblasts express an external membrane glycoprotein of 145,000 $M_r$ (T145) only after activation. A subsequent study, however, found this SDS-PAGE band lacking on peritoneal exudate lymphocytes with high cytolytic activity (42).

Gately (43) found an intensely-labeled band of 11,000 $M_r$ (T11) on SDS-PAGE of purified plasma membranes of [$^{35}$S]methionine internally-labeled CTL-rich lymphocytes. The band was present at lower specific activity in the endoplasmic reticulum fraction, and was virtually absent from the cytosol or nuclei-mitochondria fractions, indicating that it is located primarily in the plasma membrane.

T11 was prominent on the T cells in primary or secondary allo-specific CTL-containing lymphocyte populations generated *in vitro* and on Concanavalin A-induced lymphoblasts, but absent or nearly absent from normal splenocytes, LPS-induced B lymphoblasts, or tumor cells such as P815 mastocytoma or the T lymphoma EL4 (Table I). Most interestingly, T11 was absent or nearly absent from PHA-induced lymphoblasts. This latter population contained 30% T lymphoblasts (the same proportion as the allo-specific CTL populations) but lacked lectin-dependent cytolytic activity. Thus, T11 expression appears to be restricted to a subpopulation of activated T lymphocytes, quite possibly CTL.

TABLE I. T11 Distribution

| Cell Type | Thy-1$^+$ | T cell Blasts | T11 | Lytic Activity |
|---|---|---|---|---|
| 1° MLC CTL | 80% | 30% | ++++ | ++ |
| 2° MLC CTL | 80 | 30 | ++++ | ++ |
| Con A Blasts | 90 | 80 | ++ | + |
| PHA Blasts | 90 | 30 | ± | - |
| Normal Spleen | 60 | 0 | - | - |
| LPS Blasts[b] | 0 | 0 | - | - |
| 1° MLC[b] | 0 | 0 | - | - |
| P815 | 0 | 0 | - | - |
| EL4 | 100 | 100 | - | - |
| RDM4 | | | ± | - |

[a]From Gately and Martz (43).

[b]Thy-1$^+$ cells removed with Ab+C.

The functions of T145 and T11 are unknown. Unless an antibody to such a molecule were to block killing, there appears to be no general approach to ascertaining the function of such a molecule. Consider, for example, our ignorance about the function of Thy-1, which was discovered seventeen years ago (44).

TABLE II. Monoclonal antibodies that block murine CTL-mediated killing in the absence of complement by binding to the CTL

| Antigen on CTL | $M_r$(s) of reduced antigen x $10^{-3}$ | References |
|---|---|---|
| Lyt-2,3 | 30, 35 | 45, 59-61, 64, 65, 73-75 |
| LFA-1 | 95, 180 | 45, 63, 68, 69, 76, 77 |

TABLE III. Monoclonal antibodies which do not block murine CTL-mediated killing when directed towards the CTL

| Antigen on CTL | $M_r$(s) of reduced antigen x $10^{-3}$ | References |
|---|---|---|
| B2-microglobulin | 12 | 46, 78 |
| Thy-1 | 25 | 45, 46, 59, 74, 79 |
| Ia | 28, 35 | 45, 46 |
| H-2 | 45 | 59, 80, 81, reviewed in 9 |
| TL | 50 | 59 |
| Lyt-1 | 70 | 45, 59 |
| Lgp-100 | 100 | 45 |
| Common leucocyte antigen (T200, Ly 5) | 200 | 45 |
| 9 misc. antigens defined by monoclonal xenoantibodies | 46, 60, 115 140, 250 | 45 |
| Immunoglobulins | | reviewed in 9 |
| Ly-6 | | 45 |
| Misc. murine leukemia virus glycoproteins | | 59 |
| Numerous misc. alloantisera | | 60 |
| Misc. xenoantisera against mouse CTL | | 82, 83 as elaborated on page 216 in 46 |
| Anti-idiotypic antisera* | | 84-87, page 140 in 88 |

*All had demonstrated activity on other T cell functions.

MONOCLONAL ANTI-CTL ANTIBODIES WHICH BLOCK KILLING

Recently, it has been demonstrated that CTL-mediated killing can be blocked with monoclonal antibodies (MAbs) against either of two molecular species on the CTL membrane in the absence of complement (Table II). This represents the first breakthrough in identifying molecules likely to play an essential role in CTL function. Screening for MAbs which block CTL-mediated killing now provides a systematic approach towards linking specific molecules with CTL functions.

The fact that certain antibodies block killing by binding to the CTL is remarkable in light of past failures to find such antibodies, summarized in Table III. Antibodies to more than a dozen distinct molecules expressed on CTLs fail to block killing (45 and Table III). This comparison is what distinguishes Lyt-2,3 and LFA-1 as being uniquely involved in CTL function.

Previously, six papers have reported blocking of murine CTL-mediated killing with conventional antisera thought to act on the CTL. In three cases, however, action on the target cell was not rigorously excluded (46-48). In the remaining three cases (49-51), action via the target cell was excluded but the xenoantisera employed recognized multiple specificities, making identification of the crucial molecules impractical. Blocking monoclonal antibodies avoid this problem since they usually bind to a single molecular species.

Human CTLs have been most consistently blocked with OKT3 MAb (52-54, but see 55). The murine homolog of the human molecule recognized by OKT3 is not clear. Blocking has also been found with anti-Leu-2a MAb (56), which appears to recognize the human homolog of Lyt-2,3 (57). The OKT5 and OKT8 MAbs, which may recognize the same molecule, have produced weaker blocking (52) but not consistently (52-54).

Some caution should be used in categorizing CTL molecules, or the MAbs which recognize them, as "blocking" or "non-blocking." We have seen that the 53.6 anti-Lyt-2 MAb of Ledbetter and Herzenberg (58) is a potent blocker of CTL-mediated killing, while the M5/24 anti-Lyt-2,3 MAb we developed (45) is a relatively poor blocker. The reason for this difference has not yet been resolved; possibilities include differences in avidity or in the exact position of the determinant recognized on the Lyt-2,3 molecule. A similar situation exists for the anti-Leu-2a and anti-leu-2b MAbs in the human CTL system (56). Clearly, the fact that one or more MAbs to a given molecule fail to block CTL-mediated killing does not preclude later discovery of a MAb to the same molecule which blocks well, or vice versa. Moreover, it is conceivable that certain pairs or trios of pooled MAbs may block while no individual member of the group is able to block by itself.

MECHANISM OF BLOCKING WITH ANTI-LYT-2,3 AND THE ROLE OF THE LYT-2,3 MOLECULE

In 1979, two groups independently discovered that antibodies to Lyt-2,3 block killing (59,60). Action via the CTL was established by using target cells genetically incapable of expressing the relevant alloantigen. Such antibodies block the formation of shear-resistant CTL-target conjugates (61), and thus act on the recognition-adhesion step. Moreover, Fan, Ahmed and Bonavida (61) obtained two kinds of direct evidence suggesting that the 53.6 anti-Lyt-2 MAb of Ledbetter and Herzenberg (58) does not act on the lethal hit. First, they showed that the number of target cells which are killed, as a percentage of those bound to CTLs in isolated binary conjugates, is not reduced by anti-Lyt-2 MAb (although the MAb reduced the number of conjugates which formed). Second, addition of MAb after formation of conjugates (and their dispersion in dextran-containing medium to prevent recycling) such that the lethal hit occurred in the presence of MAb did not reduce subsequent $^{51}Cr$-release.

In a more recent study, Shinohara and coworkers (62), using a calcium pulse analysis, have confirmed that mouse allo-anti-Lyt-2 blocks the recognition-adhesion step. Moreover, they provide evidence that anti-Lyt-2 reverses already completed recognition-adhesion events within 5 minutes. The same study (62) also noted that for effective blocking, the antibody must be present during CTL-target interaction. CTL which were pretreated and rinsed were not blocked. (We have made similar observations with M7/14, but the story is apparently more complicated, see 63.)

MacDonald and coworkers (64) have shown that H-$2^b$ anti-H-$2^d$ CTL clones differ considerably in their susceptibility to blocking by anti-Lyt-2 (MAb 53.6). These differences are not accounted for by differences in density of Lyt-2 expression on the CTLs. It seems likely that high avidity clones are less inhibited than low avidity clones.

Ledbetter and coworkers have presented evidence that the Lyt-2 and Lyt-3 antigenic determinants are carried on separate disulfide-bonded polypeptides (65). They found that selective removal of Lyt-3 with trypsin, which left over 60% of Lyt-2 intact, affected the killer activity of various CTL populations differently. In one case where removal of Lyt-3 left substantial killing activity, killing was less inhibited by anti-Lyt-3 and more inhibited by anti-Lyt-2 antibodies.

Fan and Bonavida (66) treated CTLs more extensively with trypsin until Lyt-2 was removed. Under these conditions, H-2, Thy-1, and some Lyt-1 remained. Nevertheless, the trypsinized CTL lost specific killing ability and specific conjugate-forming ability. Incubation of the trypsinized CTLs at 37°C for 3 hours allowed about 50% recovery of Lyt-2 expression, killing, and conjugate-forming ability.

Dialynas, Glasebrook and Fitch (67) treated CTL clones with the mutagen ethyl methane sulfonate and selected for antigen-loss variants. A clone which no longer expressed a monoclonal Thy-1.2 determinant retained specific killing activity. However, a clone which no longer expressed several monoclonally defined Lyt-2,3 determinants lost the ability to kill specifically. Non-specific Con A-dependent killing ability was retained, however, showing that the Lyt-2,3 antigens were not essential for lethal hit delivery. The question of whether the variant clones lost the entire molecule or only lost certain antigenic determinants while continuing to express the molecule remains open.

These data (66,67) suggest that the Lyt-2,3 molecule may be essential for specific recognition.

## IDENTIFICATION OF LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN ONE (LFA-1)

In 1979, we began screening for monoclonal blocking antibodies. We immunized rats with mouse CTLs. We hoped that xenoimmunization would increase the number of immunogenic CTL molecules; this may have been the case since no alloantibodies to LFA-1 have been reported. Secondly, we wanted to avoid antibodies to the target cell. For this purpose, we used mouse anti-rat CTLs as immunogen in the rat (a "doubly xenogeneic" protocol), and then studied the blocking of the

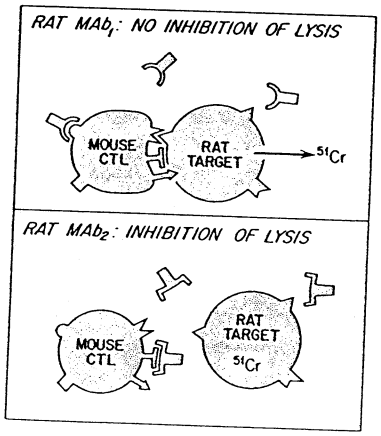


Fig. 1. Doubly xenogeneic rationale for development of blocking monoclonal antibodies. Rat MAbs are tested for blocking on a mouse CTL anti-rat lymphoma target cell system. Antibodies which might block by binding to the target are avoided since the target cell is syngeneic with the rat antibody donor.

mouse anti-rat killing by the resulting rat anti-mouse antibodies (Fig. 1). Both the donor of the antibody and the $^{51}Cr$-labeled assay target were thus of inbred Lewis rat genotype (45). The hybridomas were rat-mouse fusions made with the non-secreting mouse myeloma derivative NSI.

Screening was also conducted for T lymphocyte-specific binding, and this was actually the basis for the decision to clone a group of hybridomas which turned out to include one producing antibody with potent CTL-blocking activity. This antibody, designated M7/14, was unique among 24 monoclonal antibodies tested for blocking. These antibodies distinguished at least 14 different molecules on the CTL membrane; only M7/14 and two anti-Lyt-2,3 antibodies blocked killing (45 and Table II).

M7/14 produced an $IgG2_a$ which immunoprecipitates two non-covalently linked polypeptide chains of 95,000 and 180,000 $M_r$ (45,68; Kürzinger, Ho, and Springer, submitted for publication). The molecule recognized by M7/14 was designated lymphocyte function-associated antigen one (LFA-1, 45) and was shown to be distinct from previously described lymphocyte membrane molecules. The molecular weights of LFA-1 clearly distinguish it from T11 (43) and T145 (41), and direct comparison on SDS-PAGE (68) shows it to be distinct from Thy-1, H-2, Lyt-1, Lyt-2,3 and T200 (also known as the common leucocyte antigen of Ly-5). In biosynthetic labeling, both chains of LFA-1 incorporate $[^3H]$-glucosamine, suggesting that both are glycoproteins (unpublished). Pierres, Goridis, and Golstein and Fitch and coworkers have developed MAbs which block CTL-mediated killing and appear to recognize the same LFA-1 molecule (63 and articles in this volume).

The xenoantibody M7/14 recognizes lymphocytes from all 9 inbred strains of mice tested, and blocks various xenogeneic, allogeneic, and syngeneic CTL-target systems (69). Thus, the specificity of M7/14 is neither idiotypic nor allotypic.

## TISSUE DISTRIBUTION AND DENSITY OF LFA-1

LFA-1 expression is not restricted to CTL or even to T cells (68). It was found by quantitative flow cytofluorometry (FACS analysis) on 100% of thymocytes, peripheral T lymphocytes, and B lymphocytes. It is present on 80% of bone marrow leucocytes, suggesting that myeloid cells express LFA-1. Thioglycollate-induced macrophages were largely negative, erythrocytes were negative, and tissue homogenates of lung, liver, brain, and kidney did not absorb M7/14 antibody (68).

Based on fluorescence (FACS) analysis of LFA-1 density, normal splenic C57BL/6J T lymphocytes are two-thirds dim and one-third

bright. The two subpopulations differ by 4-fold in LFA-1 density (68). The dim T cells are 1.7-fold brighter than B cells, and the bright T cells, about 7-fold. The average density for pooled T lymphocytes is 3.5-fold greater than that for B cells.

Activation of T lymphocytes by allogeneic secondary MLC (to produce a population with high CTL activity) or by Con A increased the LFA-1 per cell by 5-fold compared to normal splenocytes, whereas H-2 per cell increased only 2-fold (68). The increase in LFA-1 was similar to that in Thy-1, and greater than that of Lyt-2,3 (Thy-1 and Lyt-2,3 densities were calculated for the positive subpopulations only).

Activation of B cells by LPS increased LFA-1/cell by 2.3-fold, but this increase was less than the corresponding 3.2-fold increase in H-2/cell.

In side-by-side SDS-PAGE analysis, the polypeptides immunoprecipitated by M7/14 from LPS activated B lymphocytes, Con A-activated T lymphocytes, or thymocytes were indistinguishable.

## EFFECTS OF M7/14 ANTIBODY ON NON-CTL FUNCTIONS

Experiments to determine the effects of M7/14 in various systems (69) have led us to the working hypothesis that LFA-1 is crucial in the interactions of T cells with other cells. In addition to blocking CTL-mediated killing, M7/14 MAb inhibits allogeneic and xenogeneic mixed lymphocyte culture proliferative responses, the macrophage-dependent, antigen-specific proliferation of primed T lymphocytes *in vitro*, and T cell dependent primary antibody (plaque-forming cell) responses to sheep red blood cells. On the other hand, responses of LFA-1-bearing cells (B lymphocytes) which do not involve known cellular interactions are not inhibited, showing that M7/14 is not generally inhibitory for proliferation: LPS-induced proliferation of B cells and the T cell-independent primary antibody (PFC) response to haptenated-Ficoll are not inhibited. Furthermore, addition of M7/14 to mixed lymphocyte cultures at one day or later is not inhibitory, showing that M7/14 does not block T cell proliferation *per se*.

## MECHANISM OF INHIBITION OF CTL-MEDIATED KILLING BY ANTIBODY TO LFA-1

The unlikely possibility that M7/14 is an autoreacive antibody which could bind to the rat lymphoma target cells used in the $^{51}Cr$-release assay was excluded by quantitative flow cytofluorometry (45).

The density of LFA-1 on lymphocyte populations high in CTL activity is 2.5-fold less than the density of H-2, and 10-fold less than Thy-1 (all based on quantitation with $IgG2_a$ MAbs, 68). Since MAbs to H-2 and Thy-1 do not block killing, blocking by anti-LFA-1 cannot be accounted for by a non-specific blanketing effect resulting from high antigen density. Also, the density of Lyt-2,3 is slightly greater than that of LFA-1.

Blocking of xenogeneic C57BL/6J anti BN-lymphoma killing averaged 90% in 17 experiments (45). Inhibition was not diminished even when highly active CTLs or high effector/target ratios were employed: 94% inhibition was found in 6 experiments in which corrected $^{51}Cr$-release was 88-99% in the absence of M7/14 (45). Inhibition of allogeneic killing was somewhat less (50-80%, 69). The purified immunoglobulin inhibited as well as the dialyzed hybridoma culture supernatant. Half maximal inhibition in the xenogeneic system occurred at 600 ng IgG/ml (69). M7/14 was not toxic to the effector cell (69).

The possibility that the inhibition might be a result of CTL agglutination, preventing intermixing with target cells, was ruled out by microscopic observation. Whereas M7/14 gave profound inhibition of killing with little or no agglutination, anti-Thy-2 (M5/49) agglutinated half of the lymphocytes but produced little or no inhibition of killing (69).

The formation of shear-resistant, microscopically counted CTL-target conjugates was inhibited by M7/14 (69).

## ROLE OF THE LFA-1 MOLECULE IN CTL FUNCTION

It is not excluded that M7/14 recognizes the specific antigen receptor on CTL, but this is rendered highly unlikely by the expression of LFA-1 not only on B lymphocytes and 80% of bonemarrow cells (68), but on the macrophage-like cell line P388D1 and on the mastocytoma P815 (unpublished data).

It appears unlikely that M7/14 interferes directly with the mechanism of programming for lysis, since it inhibits conjugate formation (strong adhesion formation) and this seems sufficient to account for the inhibition of killing. Although CTL-target contact in the absence of strong adhesion may permit limited killing under certain circumstances (70,71), it seems clear that the strong adhesion triggered by antigen recognition (25) greatly increases the efficiency of killing (9,70).

The most likely role for LFA-1 would thus appear to be a role in adhesion formation between T cells and other cells (target cells, and macrophages and/or B lymphocytes). This is currently under investigation. It is interesting to note that while macrophages

appear to lack LFA-1 (68), they express the Mac-1 molecule (72) which may be homologous to LFA-1. Not only are the molecular weights of the polypeptide chains quite similar between Mac-1 and LFA-1, but the smaller polypeptides in each case appear closely related or identical on the basis of recent peptide mapping (Kürzinger, Ho and Springer, submitted for publication).

## SUMMARY

During the past decade the mechanism of CTL-mediated killing has been resolved into 3 steps, and its cation requirements, and general nature have been well defined. However, biochemical understanding of the CTL-target interaction has made little progress. Recently, we have developed a monoclonal antibody (MAb) which blocks killing by binding to a previously undescribed molecule on the CTL membrane, a molecule which we therefore have termed lymphocyte function-associated antigen one (LFA-1). LFA-1 and Lyt-2,3 are the only presently identified sites for such blocking; antibodies to over a dozen other molecules expressed on the CTL do not block killing. Present evidence suggests that LFA-1 is crucial in the adhesive interaction of T cells with other cells (e.g., targets, macrophages, perhaps B cells). The continuing search for blocking MAbs provides a systematic way to link specific molecules with CTL function.

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## ABBREVIATIONS USED IN THIS PAPER

CTL, cytolytic T lymphocyte; LFA-1, lymphocyte function-associated antigen one; M7/14, the blocking monoclonal antibody (or more properly the hybridoma which secretes it) which recognizes and defines LFA-1; MAb, monoclonal antibody; $M_r$, relative molecular mass.

REFERENCES

1. Brunner, K.T., J. Mauel, J.-C. Cerottini, H. Rudolf, and B. Chapuis. In vitro studies of cellular and humoral immunity induced by tumor allografts. In "Mechanisms of Inflammation Induced by Immune Reactions," P.A. Miescher and P. Grabar, eds., pp. 342-357 (1967).
2. Berke G., G. Yagil, H. Ginsburg, and M. Feldman. Kinetic analysis of a graft reaction induced in cell culture. Immunol. 17:723-740 (1969).
3. Doherty, P.C., and R.M. Zinkernagel. T-cell-mediated immunopathology in viral infections. Transplant. Rev. 19:89-120 (1974).
4. Cerottini, J.-C., and K.T. Brunner. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. Adv. Immunol. 18:67-132 (1974).
5. Martz, E. Early steps in specific tumor cell lysis by sensitized mouse T lymphocytes. II. Electrolyte permeability increase in the target cell membrane concomitant with programming for lysis. J. Immunol. 117:1023-1027 (1976).
6. Sanderson, C.J. The mechanism of lymphocyte-mediated cytotoxicity. Biol Rev. 56:153-197 (1981).
7. Boyle, M.D.P., S.H. Ohaninan, and T. Borsos. Studies on the terminal stages of antibody-complement-mediated killing of a tumor cell. II. Inhibition of transformation of T* to dead cells by 3'5'cAMP. J. Immunol. 116:1276-1279 (1976).
8. Burakoff, S.J., E. Martz, and B. Benacerraf. Is the primary complement lesion insufficient for lysis? Failure of cells damaged under osmotic protection to lyse in EDTA or at low temperature after removal of osmotic protection. Clin. Immunol. and Immunopathol. 4:108-126 (1975).
9. Martz, E. Mechanism of specific tumor cell lysis by alloimmune T-lymphocytes: Resolution and characterization of discrete steps in the cellular interaction. Contemp. Top. Immunobiol. 7:301-361 (1977).
10. Hiserodt, J.C., G.J. Tiangco, and G.A. Granger. The LT system in experimental animals. IV. Rapid specific lysis of $^{51}Cr$-labeled allogeneic target cells by highly unstable high MW lymphotoxin-receptor complex(es) released in vitro by activated alloimmune murine T lymphocytes. J. Immunol. 123:332-341 (1979).
11. Martz, E., and B. Benacerraf. Multiple target cell killing by the cytolytic T-lymphocyte and the mechanism of cytotoxicity. Transplantation 21:5-11 (1976).
12. Zagury, D., J. Bernard, N. Thierness, M. Feldman, and G. Berke. Isolation and characterization of individual functionally reactive cytotoxic T lymphocytes: conjugation, killing and recycling at the single cell level. Eur. J. Immunol. 5:818-822 (1975).
13. Golstein, P. Sensitivity of cytotoxic T cells to T-cell mediated

cytotoxicity. Nature 252:81-83 (1974).
14. Kuppers, R.C., and C.S. Henney. Evidence for direct linkage between antigen recogntion and lytic expression in effector T cells. J. Exp. Med. 143:684-689 (1976).
15. Bubbers, J.E., and C.S. Henney. Studies on the synthetic capacity and antigenic expression of glutaraldehyde-fixed target cells. J. Immunol. 114:1126-1131 (1975).
16. Thorn R.M., and C.S. Henney. Studies on the mechanism of lymphocyte-mediated cytolysis. VI. A reappraisal of the requirement for protein synthesis during T cell-mediated lysis. J. Immunol. 116:146-149 (1976).
17. Bevan, M.J., and M. Cohn. Cytotoxic effects of antigen- and mitogen-induced T cells on various targets. J. Immunol. 114:559-565 (1975).
18. Gately, M.., and E. Martz. Comparative studies on the mechanisms of nonspecific, Con A-dependent cytolysis and specific T cell-mediated cytolysis. J. Immunol. 119:1711-1722 (1977).
19. Parker, W.L., and E. Martz. Lectin-induced nonlethal adhesions between cytolytic T lymphocytes and antigenically unrecognizable tumor cells, and nonspecific "triggering" of cytolysis. J. Immunol. 124:25-35 (1980).
20. Kuppers, R.C., and C.S. Henney. Studies on the mechanism of lymphocyte-mediated cytolysis. IX. Relationships between antigen recognition and lytic expression in killer T cells. J. Immunol. 118:71-76 (1977).
21. Grimm, E., and B. Bonavida. Mechanism of cell-mediated cytotoxicity at the single cell level. I. Estimation of cytotoxic T lymphocyte frequency and relative lytic efficiency. J. Immunol. 123:2861-2869 (1979).
22. Golstein, P., and E.T. Smith. The lethal hit stage of mouse T and non-T cell-mediated cytolysis. Differences in cation requirements and characterization of an analytical "cation pulse" method. Eur. J. Immunol. 6:31-37 (1976).
23. Wagner, H., and M. Rollinghoff. T cell-mediated cytotoxicity: Discrimination between antigen recognition, lethal hit and cytolysis phase. Eur. J. Immunol. 4:745-750 (1974).
24. Martz, E. Early steps in specific tumor cell lysis by sensitized mouse T-lymphocytes. I. Resolution and characterization. J. Immunol. 115:261-267 (1975).
25. Martz, E. Immune lymphocyte to tumor cell adhesion: magnesium sufficient, calcium insufficient. J. Cell Biol. 84:584-598 (1980).
26. Gately, M.K., and E. Martz. Early steps in specific tumor cell lysis by sensitized mouse T lymphocytes. III. Resolution of two distinct roles for calcium in the cytolytic process. J. Immunol. 122:482-489 (1979).
27. Plaut, M., J.E., Bubbers, and C.S. Henney. Studies on the mechanism of lymphocyte-mediated cytolysis. VII. Two stages in the T cell-mediated lytic cycle with distinct cation requirements. J. Immunol. 116:150-155 (1976).

28. Golstein, P., C. Foa, and I.C.M. MacLennan. Mechanism of T-cell-mediated cytolysis: The differential impact of cytochalasins at the recognition and lethal hit stages. Eur. J. Immunol. 8:302-309 (1978).
29. Gately, M.K., and E. Martz. Inhibition of the lethal hit phase of T cell-mediated cytolysis by pharmacologic agents. Fed. Proc. 38:1166 (Abs. #4960) (1979).
30. Golstein, P. and E.T. Smith. Mechanism of T cell-mediated cytolysis: The lethal hit stage. Contemp. Top. Immunobiol. 7:273-300 (1977).
31. Martz E., C.D. Tsoukas, and W.J. Wechter. Evidence against $Ca^{++}$ poisoning by killer cells: Mast cells killed by T lymphocytes do not secrete prelytically. J. Supramol. Struct. Suppl. 3:311 (Abstract 818) (1979).
32. Ko, L., and D. Lagunoff. Depletion of mast cell ATP inhibits complement-dependent cytotoxic histamine release. Exp. Cell Res. 100:313-321 (1976).
33. Henney, C.S. T cell mediated cytolysis: Consideration of the role of a soluble mediator. J. Reticuloendothelial Soc. 17:231-235 (1975).
34. Todd, R.F., and G. Berke. Functional characterization of membrane components of cytotoxic peritoneal exudate T lymphocytes. I. Search for T cell receptor activity in lymphocyte membrane fractions. Immunochemistry 11:313-320 (1974).
35. Whisnant, C.C., K.H. Singer, and D.B. Amos. Interaction of cytotoxic T lymphocytes with target cells. I. Specific inhibition by detergent-solubilized, partially purified mouse histocompatibility anitgens. J. Immunol. 121:2253 (1978).
36. Todd, R.F. III, R.D. Stulting, and D.B. Amos. Lymphocyte-mediated cytolysis of allogeneic tumor cells in vitro. I. Search for target antigens in subcellular fractions. Cellular Immunol. 18:304-323 (1975).
37. Nagy, Z.A., and B.E. Elliott. The receptor specificity of alloreactive T cells. Distinction between stimulator K, I, and D region products and degeneracy of third-party H-2 recognition by low-affinity T cells. J. Exp. Med. 150:1520-1537 (1979).
38. Linna, T.J., H.D. Engers, J.-C. Cerottini, and K.T. Brunner. Inhibition of cytolytic T lymphocyte activity with subcellular alloantigen preparations and with unlabeled allogenic target cells. J. Immunol. 120:1544-1549 (1978).
39. Gilmer, P.J., H.O. McDevitt, and H.M. McConnell. Inhibition of specific T cell-target cell conjugates by target cell plasma membranes. J. Immunol. 120:774 (1978).
40. Ozato, K., H.K. Ziegler, and C.S. Henney. Liposomes as model membrane systems for immune attack. I. Transfer of antigenic determinants to lymphocyte membranes after interactions with hapten-bearing liposomes. J. Immunol. 121:1376 (1978).
41. Kimura, A.K., and H. Wigzell. Cell surface glycoproteins of murine cytotoxic T lymphocytes. I. T 145, a new surface

glycoprotein selectively expressed on Ly $1^{-}2^{+}$ cytotoxic T lymphocytes. J. Exp. Med. 147:1418-1434 (1978).

42. Berke, G., Y. Kaufmann, and D. Gabison. Cell surface determinants of cytotoxic T lymphocytes: possible role in lymphocyte-target cell interaction. Fed. Proc. 39:1198 (Abstract #4891) (1980).
43. Gately, M.K., and E. Martz. T11: A new protein marker on activated murine T lymphocytes. J. Immunol. 126:709-714 (1981).
44. Reif, A.E., and J.M. Allen. The AKR thymic antigen and its distribution in leukemias and nervous tissues. J. Exp. Med. 120:413 (1964).
45. Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T.A. Springer. Lymphocyte function-associated antigen one (LFA-1): a surface antigen distinct from Lyt-2/3 that participates in T lymphocyte-mediated killing. Proc. Natl. Acad. Sci. USA 78:4535-4539 (1981).
46. Kimura, A.K., and H. Wigzell. Cytotoxic T lymphocyte membrane components: An analysis of structures related to function. Cont. Top. Mol. Immunol. 6:209-244 (1977).
47. Redelman, D., and P.E. Trefts. In vitro studies of the rabbit immune system. VIII. Rabbit anti-mouse cytotoxic T-effector cells are inhibited by anti-rabbit T-cell serum in the absence of complement. J. Immunol. 121:1532-1539 (1978).
48. Rabinowitz, R., and M. Schlesinger. Inhibition of the activity of cytotoxic murine T lymphocytes by antibodies to idiotypic determinants. Immunol. 39:93-99 (1980).
49. Kimura, A.K. Inhibition of specific cell-mediated cytotoxicity by anti-T-cell receptor antibody. J. Exp. Med. 139:888-901 (1974).
50. Rabinowitz, R., R. Laskov, and M. Schlesinger. Inhibition of cell-mediated lysis by xenoantibodies reactive with effector T lymphocytes. Eur. J. Immunol. 10:219-223 (1980).
51. Hiserodt, J.C., and B. Bonavida. Studies on the induction and expression of T cell-mediated immunity. XI. Inhibition of the "Lethal Hit" in T cell-mediated cytotoxicity by heterologous rat antiserum made against alloimmune cytotoxic T lymphocytes. J. Immunol. 126:256-262 (1981).
52. Platsoucas, C.D., and R.A. Good. Inhibition of specific cell-mediated cytotoxicity by monoclonal antibodies to human T cell antigens. Proc. Natl. Acad. Sci. USA 78:4500-4504 (1981).
53. Chang, T.W., P.C. Kung, S.P. Gingras, and G. Goldstein. Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? Proc. Natl. Acad. Sci. USA 78: 1805-1808 (1981).
54. Tsoukas, C.D., R.I. Fox, D.A. Carson, S. Fong, and J.H. Vaughan. Monoclonal antibody OKT3 blocks the function of human cytotoxic T-lymphocytes against autologous EBV-transformed target cells. Submitted for publication.
55. Reinherz, E.L., R.E. Hussey, and S.F. Schlossman. A monoclonal antibody blocking human T cell function. Eur. J. Immunol. 10:

758-762 (1980).

56. Evans, R.L., D.W. Wall, C.D. Platsoucas, F.P. Siegal, S.M. Fikrig, C.M. Testa, and R.A. Good. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to $T_{H2}$ antigen. Proc. Natl. Acad. Sci. USA 78:544-548 (1981).
57. Ledbetter, J.A., R.L. Evans, M. Lipinski, C. Cunningham-Rundles, R.A. Good, and L.A. Herzenberg. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J. Exp. Med. 153:310-323 (1981).
58. Ledbetter, J.A., and L.A. Herzenberg. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunological Rev. 47:63-89 (1979).
59. Nakayama, E., H. Shiku, E. Stockert, H.F. Oettgen, and L.J. Old. Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. Proc. Natl. Acad. Sci. USA 76:1977-1981 (1979).
60. Shinohara, N., and D.H. Sachs. 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. 150:432-444 (1979).
61. Fan, J., A. Ahmed, and B. Bonavida. 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 nonspecific cytotoxicity: Evidence for the blocking of the binding between T lymphocytes and target cells and not the post-binding cytolytic steps. J. Immunol. 125: 2444-2453 (1980).
62. Shinohara, N., M. Taniguchi, and M. Kojima. Mouse alloantibodies capable of blocking cytotoxic T cell function. III. Studies of the mechanism of blocking of CML by anti-Lyt-2 antibodies. J. Immunol. 127:1575-1578 (1981).
63. Pierres, P., C. Goridis, and P. Golstein. Inhibition of murine T cell-mediated cytolysis and T cell proliferation by a rat monoclonal antibody immunoprecipitating two lymphoid cell surface polypeptides of 94,000 and 180,000 molecular weight. Eur. J. Immunol., in press (1981).
64. MacDonald, H.R., N. Thiernesse, and J.-C. Cerottini. Inhibition of T cell-mediated cytolysis by monoclonal antibodies directed against Lyt-2: heterogeneity of inhibition at the clonal level. J. Immunol. 126:1671-1675 (1981).
65. Ledbetter, J.A., W.E. Seaman, T.T. Tsu, and L.A. Herzenberg. Lyt-2 and Lyt-3 antigens are on two different polypeptide subunits linked by disulfide bonds. Relationship of subunits to T cell cytolytic activity. J. Exp. Med. 153:1503-1516 (1981).
66. Fan, J., and B. Bonavida. Studies on the induction and expression of T cell-mediated immunity. XII. The concomitant loss and recovery of membrane-associated Lyt-2 antigens, lymphocyte-target cell binding, and the antigen-specific and -nonspecific

cytotoxic activity of alloimmune T lymphocytes after treatment with trypsin. J. Immunol. 127:1856-1864.(1981).

67. Dialynas, D.P., M.R. Loken, A.L. Glasebrook, and F.W. Fitch. Lyt-2$^-$/Lyt-3$^-$ variants of a cloned cytolytic T cell line lack an antigen receptor functional in cytolysis. J. Exp. Med. 153:595-604 (1981).
68. Kurzinger, K., T. Reynolds, R.N. Germain, D. Davignon, E. Martz., and T.A. Spjringer. A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. J. Immunol. 127:596-602 (1981).
69. Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T.A. Springer. Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1). Mechanism of blockade of T lymphocyte-mediated killing and effects on other T and B lymphocyte functions. J. Immunol. 127:590-595 (1981).
70. Shortman, K., and P. Golstein. Target cell recognition by cytolytic T cells: Different requirements for the formation of strong conjugates or for proceeding to lysis. J. Immunol. 123: 833-839 (1979).
71. Martz, E. Inability of EDTA to prevent damage mediated by cytolytic T-lymphocytes. Cellular Immunol. 20:304-314 (1975).
72. Springer, T.A. Mac-1,2,3, and 4: Murine macrophage differentiation antigens identified by monoclonal antibodies. In: "Heterogeneity of mononuclear phagocytes," O. Forster, ed., Academic Press, New York. In press (1980).
73. Shinohara, N., U. Hammerling, and D.H. Sachs. Mouse alloantibodies capable of blocking cytotoxic T cell function. II. Further study on the relationship between the blocking antibodies and the products of the Lyt-2 locus. Eur. J. Immunol. 10:589-594 (1980).
74. Hollander, N., E. Pillemer, and I.L. Weissman. The blocking effect of Lyt-2 antibodies on T cell functions. J. Exp. Med. 152:674-687 (1980).
75. Sarmiento, M., A.L. Glasebrook, and F.W. Fitch. IgG or IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt-2 antigen block T cell-mediated cytolysis in the absence of complement. J. Immunol. 125:2665-2672 (1980).
76. Springer, T.A., K. Kurzinger, T. Reynolds, R.N. Germain, D. Davignon, and E. Martz. Monoclonal antibodies as probes of surface structures participating in T lymphocyte function. In: "Monoclonal Antibodies and T Cell Hybridomas," U. Hammerling, G. Hammerling, and J. Kearney, eds., Elsevier. In press (1981).
77. Golstein, P., and M. Pierres. Monoclonal antibodies as probes to study the mechanism of T cell-mediated cytolysis. Proc. 14th Leukocyte Cult. Conf., in press (1981).
78. Lightbody, J.J., L. Urbani, and M.D. Poulik. Effect of beta-2 microglobulin antibody on effector function of T-cell mediated cytotoxicity. Nature 250:227-228 (1974).

79. Golstein, P. H. Wigzell, H. Blomgren, and E.A.J. Svedmyr. Cells mediating specific in vitro cytotoxicity. II. Probable autonomy of thymus-processed lymphocytes (T cells) for the killing of allogeneic target cells. J. Exp. Med. 135:890-906 (1972).
80. Lindah., K.F., and H. Lemke. Inhibition of killer-target cell interaction by monoclonal anti-H-2 antibodies. Eur. J. Immunol. 9:526-536 (1979).
81. Epstein, S.L., K. Ozato, and D.H. Sachs. Blocking of allogeneic cell-mediated lympholysis by monoclonal antibodies to H-2 antigens. J. Immunol. 125:129-135 (1980).
82. Rothstein, T.L., M.G. Mage, J. Mond, and L.L. McHugh. Guinea pig antiserum to mouse cytotoxic T lymhocytes and their precursors. J. Immunol. 120:209 (1978).
83. Sullivan, K.A., G. Berke, and D.B. Amos. An antigenic determinant of cytotoxic lymphocytes. Transplantation 16:388-391 (1973).
84. Braun, M. and F. Saal. The T-cell receptor and cytotoxicity. An anti-idiotype antiserum that inhibits a graft-versus-host reaction does not inhibit cell-mediated cytotoxicity. Cellular Immunol. 30:254-260 (1977).
85. Lindahl, K.F. Antisera against recognition sites: lack of effect on the mixed leucocyte culture interaction. Eur. J. Immunol. 2:501-504 (1972).
86. Sherman, L.A., S.J. Burakoff, and B. Benacerraf. The induction of cytolytic T lymphocytes with specificity for p-azophenylarsonate coupled syngeneic cells. J. Immunol. 121:1432 (1978).
87. Rubin, B., P. Golstein, O. Nordfang, and B. Hertel-Wulff. Generation of H-2-reactive T cell lines that bear the 5936 idiotype(s). J. Immunol. 124:161-167 (1980).
88. Binz, H., and H. Wigzell. Antigen-binding, idiotypic T-lymphocyte receptors. In: "Contemporary topics in immunobiology volume 7: T cells," O. Stutman, Ed., Plenum Press, New York, pp. 113-177 (1977).
89. Glasebrook, A.L. Conjugate formation by primary and secondary populations of murine immune T lymphocytes. J. Immunol. 121: 1870-1877 (1978).