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SUSCEPTIBILITY OF CYTOTOXIC T LYMPHOCYTE (CTL) CLONES TO INHIBITION BY ANTI-T3 AND ANTI-T4 (BUT NOT ANTI-LFA-1) MONOCLONAL ANTIBODIES VARIES WITH THE "AVIDITY" OF CTL-TARGET INTERACTION

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To explore the role of the T3, T4, and LFA-1 molecules in high and low "avidity" interactions between SB2-specific cytotoxic T lymphocyte (CTL) clones and their targets, monoclonal antibody-mediated inhibition of cytotoxicity has been studied in experiments that vary the "avidity" of interaction in three different ways. 1) Previous results have been extended with respect to different CTL clones assayed on the same SB2-positive target cells. Differences between clones in susceptibility to anti-T3 inhibition paralleled variations in anti-T4 inhibition, and both correlated inversely with the "avidity" of the effector-target interaction (inferred previously from studies of conjugate dissociation). 2) A high "avidity" clone, 8.4, was identified that lysed not only SB2-positive cells but also cross-reacted on a few SB2-negative cells. Cold target inhibition studies confirmed the cross-reaction, and together with conjugate dissociation studies, indicated that cross-reaction to be of lower "avidity" than the specific recognition of SB2. Cross-reactive lysis was much more susceptible to inhibition by anti-T3 and anti-T4 than was specific lysis. 3) Anti-T3 and anti-T4 blocking was analyzed in the presence of anti-Ia antibody to reduce the amount of Ia antigen available on the target. Anti-T3 and anti-T4 antibody blocking was more efficient after the addition of anti-Ia antibody concentrations that (by themselves) produced minimal inhibition of lysis. As a control, anti-LFA-1 antibody blocking was analyzed in each of these three experimental systems that compare interactions of different "avidity"; minimal variation was observed in the efficiency of inhibition by anti-LFA-1. Thus, anti-T3 and anti-T4 inhibition correlates inversely with the "avidity" of that CTL-target interaction, but anti-LFA-1 inhibition does not.

The details of T cell recognition of antigen on cell membranes remain obscure. However, identification of the cell surface molecules involved in this process has been facilitated by analysis of monoclonal antibody-mediated inhibition of T cell function. Among the three molecules whose function is analyzed in the present study, two are thought to be involved in the formation of conjugates between cytotoxic T lymphocytes (CTL) and targets: T4 (Leu-3) (1, 2) and LFA-1 (3). Evidence that the T4 molecule may be functionally involved in T cell recognition derives largely from findings that the T4 molecule is found almost exclusively on T cells that recognize class II (rather than class I) major histocompatibility complex (MHC) antigens (4–7), and antibodies to the T4 molecule inhibit antigen-specific proliferative responses (8, 9), antigen-specific cell-mediated lysis (CML)[1] (7, 10, 11), and antigen-specific effector-target conjugation (2). These results have given rise to the hypothesis that the T4 molecule interacts with a nonpolymorphic portion of class II molecules and facilitates the binding of Ia-bearing cells by T4-positive T cells (7, 12).

The LFA-1 molecule, unlike T4, has a wide tissue distribution, including cells outside the lymphoid system (13–15). Antibodies against the LFA-1 molecule inhibit many functions of T cells—CML (13, 14), antigen-specific T cell proliferation (13), and lymphokine secretion.[2] LFA-1 has been ascribed the function of an adhesive molecule that facilitates intracellular aggregation, because antibodies against LFA-1 inhibit aggregation of activated T cells (16) and of lymphoblastoid B cell lines (LCL) (E. Martz and J. Hildreth, personal communications).

In contrast to T4 and LFA-1, the T3 (Leu-4) molecule blocks the CML response during the process that follows conjugate formation (17, 18). Soluble antibodies specific for this molecule inhibit CML (19–22) and proliferation (23, 24). Moreover, soluble antibodies (in the presence of accessory cells) or antibody coupled on a solid support induce T cell mitogenesis (19, 25, 26) and lymphokine secretion (26, 27). Furthermore, the T3 molecule is part of a molecular complex on the cell surface that is believed to include the antigen-specific receptor (Ti); this complex of molecules is modulated after treatment either with anti-Ti or anti-T3 antibody (28), or after in vitro tolerance induction (29). These data suggest that the T3 molecule may be involved in transducing a signal from the antigen-specific receptor (reviewed in Reference 30).

Recently, it has been observed that there are differences between human clones in their susceptibility to inhibition by antibodies against T3 and T4 (1, 31). In

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[1] Abbreviations used in this paper: CML, cell-mediated lysis; LCL, lymphoblastoid B cell line.

[2] **Golde, W. T., J. W. Kappler, J. Greenstein, B. Malissen, L. Hood, and P. Marrack.** 1984. The MHC-restricted antigen receptor on T cells. VIII. The role of the LFA-1 product. *Submitted for publication.*

addition, analysis of murine hybridomas has shown differences in suspectibility to inhibition by anti-LFA-1.[2] These findings have raised the possibility that there are differences between clones in the functional role of these molecules. Such speculation must be considered in light of the only established structural difference between $T4^+$ CTL clones—namely, their antigen-specific receptor (Ti) (32–34). We assume that this structure is used in a functionally similar manner by all CTL clones, and that variations in this structure will largely dictate differences in the "avidity" of interaction of different clones on the same target. Other molecules involved in the interaction are often referred to as "accessory" molecules. It is not known whether all of them (e.g., T4, T8, T11/LFA-2, LFA-1, and LFA-3) are required for each CTL-target interaction; indeed it has been widely discussed that some of them (T4, T8, LFA-1) may be required only in "low 'avidity'" interactions, which they would strengthen and facilitate (1, 31, 35).[2] However, when the logic used to interpret the anti-T4 inhibition data is applied to the data on anti-T3 inhibition, it leads to the conclusion that triggering of some clones can occur independently of the T3 molecule (31). Because this requires postulating an additional triggering mechanism, it does not seem parsimonious. Consequently, we have reevaluated this logic, and have used three different approaches involving antibody inhibition to evaluate the relationship between "avidity" of the CTL-target interaction and its susceptibility to inhibition by anti-T3, anti-T4, and anti-LFA-1 antibodies.

MATERIALS AND METHODS

Human blood reagents. Peripheral blood mononuclear leukocytes (PBL) and plasma were obtained by batch leukapheresis of normal adult volunteers (36). PBL were separated by flotation on Ficoll-Hypaque and were cryopreserved as described (36). Plasma from five to 10 male donors were pooled, frozen in aliquots, and used as a normal human plasma pool. HLA serotyping of cells was performed by the Blood Center of Southeastern Wisconsin, Milwaukee, WI. SB typing was performed by primed lymphocyte typing as described (37).

Immunochemical reagents. The monoclonal antibodies and their purification were generally as described (1). In addition, an anti-LFA-1 antibody TS1/22 (IgG1) (38) was used as Ig purified from ascites by protein A affinity chromatography. Two anti-Ia antibodies were used: SG171 (IgG2), a broadly reactive anti-Ia antibody (39), was kindly provided by Dr. J. Silver (Hospital for Joint Diseases, New York) as a mouse ascites fluid; hybridoma B7/21 (IgG2) (40), which produces an SB-specific monoclonal antibody, was kindly provided by Dr. I. Trowbridge (Salk Institute, La Jolla, CA), and the antibody used as culture supernatant.

SB-specific CTL clones. The generation and maintenance of the SB-specific CTL clones were generally as described (1). In brief, cryopreserved clones were thawed and then were stimulated with irradiated SB2-positive stimulator cells in media consisting of RPMI 1640 with 10% human plasma, penicillin, and streptomycin. The next day, phytohemagglutinin-induced T cell supernatant (1) was added (15% by volume). Cytotoxicity was assayed after 4 to 6 days of incubation of cells at 37°C in 6% CO_2-air.

Cytotoxic assays. The standard ^{51}Cr-release assay was performed as described (36). Target cells were LCL transformed with Epstein-Barr virus (36). In antibody blocking assays, effector cells were generally preincubated with antibodies for 15 to 20 min at 37°C before addition of target cells; in the experiments with limiting-dose anti-Ia inhibition, the target cells were preincubated with anti-Ia while the effectors were preincubated with anti-effector antibodies. For cold target competitor experiments, the unlabeled competitor cells were preincubated for 15 min with the effectors at 37°C before addition of the labeled target cells.

Conjugate dissociation. This assay was performed as described by Balk and Mescher (41) and as adapted previously in our lab (1). Briefly, it is a three step assay. 1) Conjugate formation: effectors and targets are allowed to form conjugates during a 5-min centrifugation at 23°C in media with 5 mM EGTA. 2) Conjugate dissociation: the pellet of cells is resuspended, competitor cells are added, and the cells are maintained in suspension with EGTA for 3 hr at 23°C. 3) Conjugate measurement: the cell suspension is transferred to media containing Ca^{++} (without EGTA, to allow lysis) and dextran (so that viscosity prevents new conjugate formation), is incubated for 4 hr at 37°C, and ^{51}Cr release is measured.

RESULTS

Variation among CTL clones in blocking by anti-T3. In our studies of monoclonal antibody inhibition of SB-specific $T3^+$, $T4^+$ CTL, we have previously emphasized heterogeneity among CTL clones in their susceptibility to inhibition by anti-T4 monoclonal antibodies (1). However, susceptibility to anti-T3 antibody inhibition also varied markedly between clones (1). Variations between clones in susceptibility to inhibition by anti-T3 correlated ($r = 0.72$, $p = 0.03$) with susceptibility to inhibition by anti-T4 (Fig. 1, panel *A*). Furthermore, the ease of dissociation of effector-target conjugates correlated with variations in anti-T3 inhibition (Fig. 1, panel *B*, $r = 0.75$, $p = 0.07$) and variations in anti-T4 inhibition (data not shown). Conjugate dissociation was measured by using an assay described by Balk and Mescher (42), and it was utilized to estimate the "avidity" of effector-target conjugates. We observed previously that the "avidity" of the interaction correlated inversely with its susceptibility to inhibition to blocking by anti-T4 antibody (1), and postulated that the T4 molecule might play a functional role in strengthening low "avidity" interactions. The data in Figure 1 illustrate that susceptibility to anti-T3 inhibition is an additional parameter that tends to correlate with the other two parameters, "avidity" and anti-T4 inhibitibility. (In addition, there is also a good correlation with susceptibility to anti-Ia inhibition, data not shown; however, because the T cell clones may not be recognizing precisely the same epitope on the SB2 molecules, we have been cautious in interpreting differences between clones in anti-Ia inhibition.)

Cross-reaction of clone 8.4 on SB2-negative targets. The foregoing data are based on analysis of different effectors on the same target. As a complementary approach, we have analyzed antibody inhibition by using the same clone of effectors on targets with which it interacts with different efficiency. These studies were made possible by the identification of cross-reactive lysis by the highest "avidity" clone, 8.4 (1). Analysis of the specificity of nine CTL clones on LCL from unrelated donors indicates that eight are strictly SB2 specific—they kill all SB2-positive donor cells, and only those cells (Sanchez-Perez et al., manuscript in preparation). The pattern of reactivity of clone 8.4 was different; it killed all SB2-positive LCL but also killed cells from several additional SB2-negative donors (Table I). There are four donors (K7, T4, PG2, and C1) whose LCL are not killed by most of the CTL clones (e.g., 8.6, 8.9, 8.10) but are killed by CTL 8.4, even at effector to target ratios less than 1:1; these LCL are subsequently referred to as "cross-reactive" targets for clone 8.4 (see below). Although none of these cells had met the SB typing criteria to be called SB2, two had elicited a weak but detectable proliferation from SB2-primed bulk populations, indicating that they triggered proliferation of a subpopulation of the T cell clones in those populations (presumably including clones like 8.4 and others).

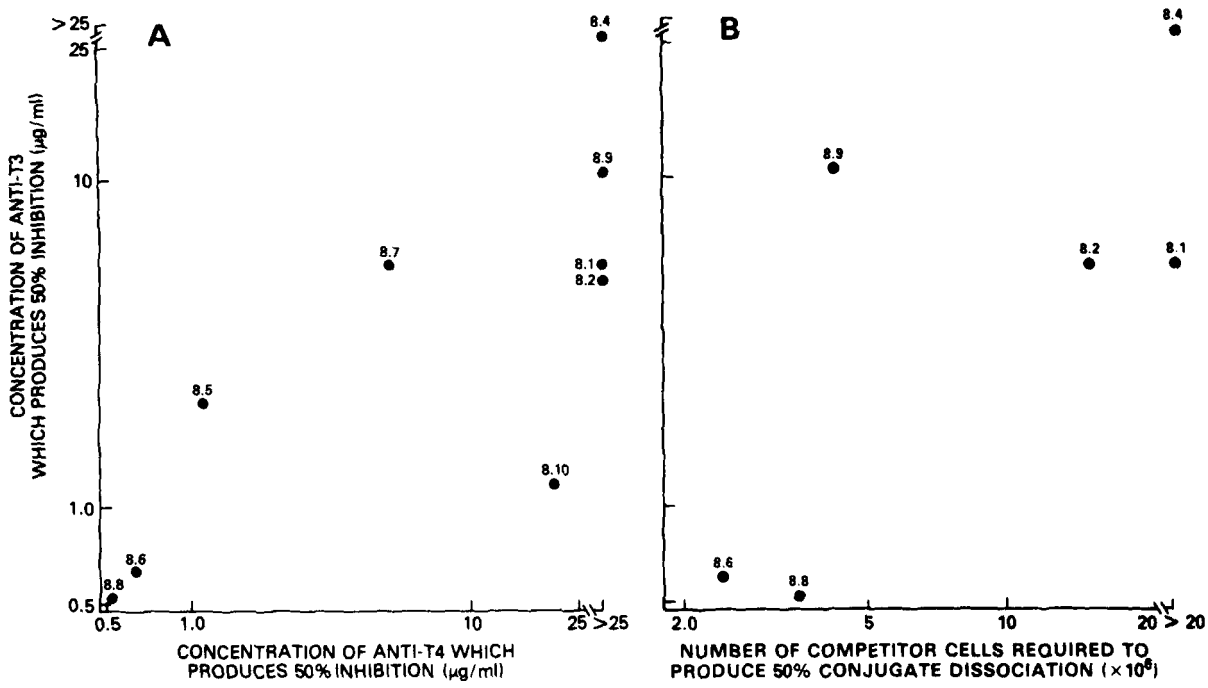

Figure 1. Correlations among a panel of CTL clones in susceptibility to blocking by anti-T3 and anti-T4 antibodies and susceptibility to conjugate dissociation. Nine clones were analyzed to determine the concentrations of OKT3 and OKT4A antibody that were required to produce a 50% decrease in the percent of lysis when present continuously during the cytotoxicity assay. Correlation of these two parameters for nine CTL clones (each represented by a *dot*) is shown in *panel A*. Six of these clones were also assayed by the technique of Balk and Mescher (42) to determine the number of unlabeled competitor cells required to cause 50% dissociation of functional effector-target conjugates (*panel B*).

TABLE I

Cytotoxic reactions of clone 8.4 on SB2-negative cells[a]

Effector Clone	E:T Ratio	Percent Lysis on Target Cells from Donor							
		B17	M16	F2	FB11	K7	T4	PG2	C1
8.6	10:1	92	92	2	0	2	0	0	0
8.9	10:1	86	84	5	6	4	6	16	0
8.10	10:1	66	57	4	0	3	3	4	0
8.4	10:1	96	90	3	2	71	38	45	55
	2:1	84				80	34	47	51
	0.7:1	80				58	23	42	43

[a] HLA phenotypes of the donors tested are as follows: B17 (A1,2, B7,8, Cw7, DR2,4, MB1,3, MT1,3); M16 (A1,3, B8,14, Cw8, DR2,3, MB1,2, MT1,2, SB1,2); F2 (A1,2, B7,8, Cw7, DR2,4, MB1,3, MT1,3, SB4,5); FB11 (A25,31, B15,40, Cw3, DR4,4, SB1,6); K7 (A2, B35,44, Cw4, DR1,8, MB1, MT1, SB?2,3); T4 (A25,30, B18,53, Cw3,4, DR4,7, MB2,3, MT 3, SB4); PG2 (A26,31, B8,35, Cw4,6, DR3,5, MB2,3, MT2, SB?2,3); C1 (A3,28, B7,56, Cw2,7, DR3,7, MB2, MT2,3, SB1).

The two simplest interpretations of the lysis by 8.4 are as follows. 1) 8.4 recognizes the same antigen on all susceptible targets; this antigen would have to be encoded by an allele of a polymorphic gene in linkage disequilibrium with SB2 because of the statistical association with SB2, but distinct from the known HLA determinants (because none of the known determinants are shared by all cells lysed; see Table I). Alternatively, 2) 8.4 recognizes the SB2 molecule but also cross-reacts on a different antigen on some other donor cells, such as the four target cells in Table I. A class II MHC molecule probably is involved in the cross-reactive lysis, because an anti-Ia monoclonal antibody (SG171; see Figure 3 below) is able to weakly inhibit lysis. Lysis of targets C1 and K7 is not inhibited by anti-SB antibody B7/21, and may therefore be independent of the SB molecule (data not shown).

Analysis by cold target inhibition. Cold target inhibition studies were performed to confirm the interaction of 8.4 with those cross-reactive targets and to determine whether they were as efficient blockers as the SB2-positive cells (Fig. 2). Lysis by clone 8.4 on the control SB2-positive target B17 (Fig. 2, panel *A*) was inhibited effectively by two SB2-positive blocker cells (B17 and M16). The cross-reactive cells K7 and C1 were also able to inhibit that lysis, although about 10-fold more of those cells were required to achieve blocking comparable with the SB2-positive cells. The profile of inhibition of the "cross-reactive" lysis of 8.4 on K7 targets (Fig. 2, panel *C*) was similar to that observed on the specific target, but much fewer inhibitor cells were required to achieve inhibition, as might be expected for a lower affinity interaction. The pattern of blocking of a control clone (clone 8.9, Fig. 2, panel *B*) was different, inasmuch as cells from donors K7 and C1 were unable to block the lysis of SB2-positive targets. These data suggest that CTL 8.4 specifically interacts with SB2-positive targets, and that its interaction with some SB2-negative targets represents a lower "avidity" cross-reaction.

Monoclonal antibody inhibition. We explored whether differences in the characteristics of specific vs cross-reactive lysis of clone 8.4 would affect the apparent functional role of the T3 and T4 molecules in the interaction. Specific lysis on the SB2$^+$ target B17 (Fig. 3, panel *A*) was resistant to inhibition by anti-T4 antibodies at 25 μg/ml, as had been observed previously (1). The cross-reactive lysis on targets K7, C1, and PG2 (Fig. 3, panels *B*, *C*, and *D*) was easily inhibited by anti-T4 antibodies. The OKT4A antibody was able to cause half-maximal inhibition of the cross-reactive lysis at 1/25th to 1/625th of that antibody concentration that was totally incapable of blocking the specific lysis on B17; even the OKT4F antibody (which is specific for the T4 molecule but less effective at inhibiting CML; W. E. B. unpublished) was able to partially inhibit lysis on targets C1 and K7.

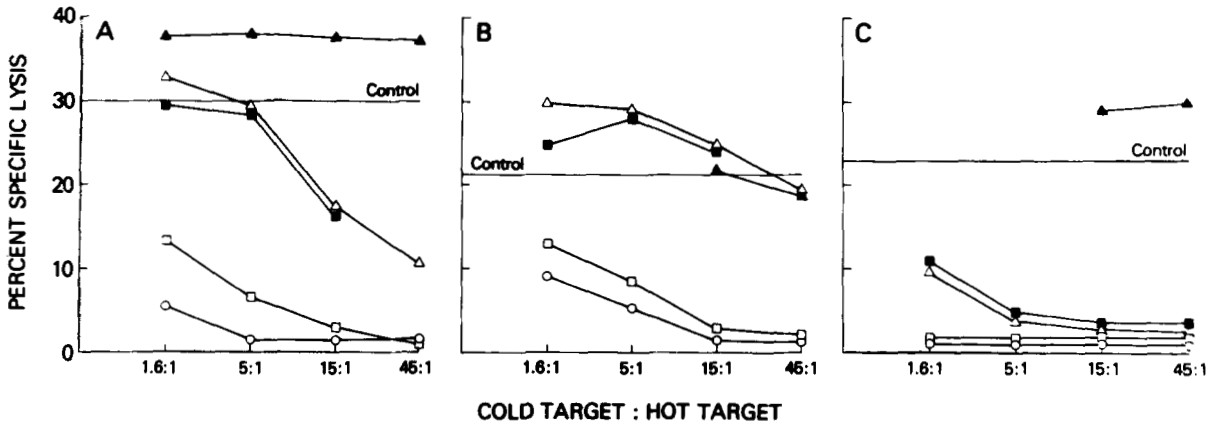

Figure 2. Cold target inhibition of specific and cross-reactive lysis. Cold target blocking was studied for the SB2-specific cytotoxicity of clone 8.4 on target B17 (*panel A*), and clone 8.9 on target B17 (*panel B*); blocking of cross-reactive lysis by clone 8.4 on target K7 is shown in *panel C*. Effectors were assayed at an E:T ratio of 1:1. Cold target inhibitor cells included SB2-positive cells (B17 □ and M16 ○) and SB2-negative cells (F2 ▲; C1 ■, and K7 △).

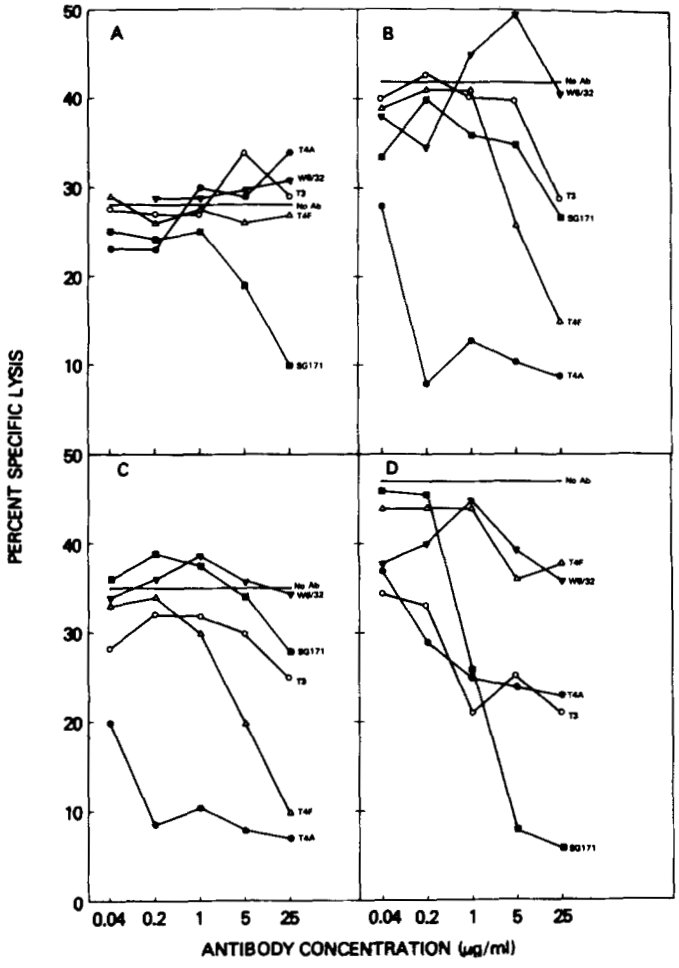

Figure 3. Monoclonal antibody inhibition of killing by clone 8.4 on four target cell populations. The cytotoxicity of clone 8.4 was assayed on an SB2-positive target (B17, *panel A*, E:T ratio 1:1) and three cross-reactive SB2-negative targets that it lyses (K7, *panel B*, E:T ratio 1:1, C1, *panel C*, E:T ratio 4:1; and PG2B, *panel D*, E:T ratio 4:1). Lysis was measured in the absence of antibody (*No Ab*) or in the presence of graded concentrations of monoclonal antibodies: anti-class I antibody w6/32 (▼), anti-T3 antibody OKT3 (○), two antibodies specific for the T4 molecule (OKT4A ●, OKT4F △), and anti-Ia antibody SG171 (■).

Clone 8.4 is also remarkable for its resistance to inhibition by antibodies against the T3 molecule (1). It is apparent (Fig. 3) that this resistance is not an invariant characteristic of the clone 8.4, but rather is characteristic of its interaction with particular targets. Its interaction with target PG2 is susceptible to inhibition by blocking by low doses of anti-T3, and some inhibition by anti-T3 is observed with the other two cross-reactions. It is intriguing that the most pronounced increase in susceptibility to anti-T4 blocking is with lysis on targets K7 and C1, whereas the most pronounced increase in susceptibility to anti-T3 blocking is with lysis on PG2.

Analysis of "avidity" of interaction by conjugate reversal. We have previously adopted an assay described by Balk and Mescher (41) to estimate the "avidity" of effector-target conjugates by dissociation in the presence of an excess of unbound competitor cells (1). To determine whether the recognition of cross-reactive targets by clone 8.4 occurs with lower "avidity", we compared the efficiency of SB2-positive cells and the cross-reactive SB2-negative cells to induce dissociation of conjugates between 8.4 and an SB2-positive target (Fig. 4). The unlabeled SB2-positive cells M16 and B17 are able to induce dissociation of conjugates between 8.4 and ^{51}Cr-labeled M16 or B17; little or no dissociation was observed in the presence of an excess of the cross-reactive cold competitors (K7 and C1). These data, together with that of cold

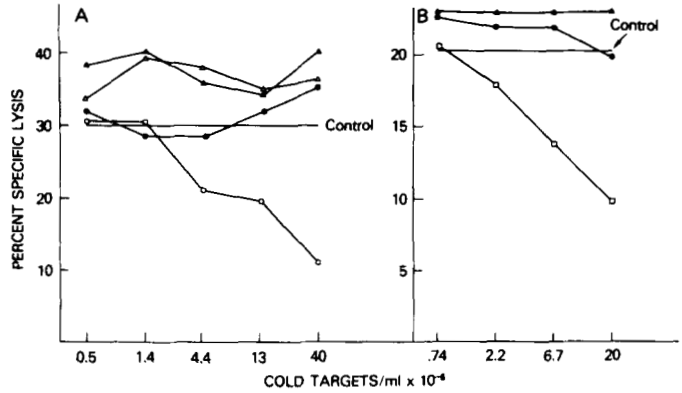

Figure 4. Inability of cross-reactive cold target competitors to dissociate conjugates of clone 8.4 on SB2-positive targets. CTL 8.4 were pelleted with labeled SB2-positive targets M16 (*panel A*) or B17 (*panel B*), and were resuspended in media that allowed conjugate formation but not lysis. To this suspension were added competitor cells from SB2-positive donors (M16 ○ and B17 □), cross-reactive SB2-negative targets (C1 △ and K7 ●), or negative control SB2-negative targets (F2 ▲), and the mixture was maintained in suspension. After 3 hr, the number of remaining functional effector-target conjugates was measured as described in *Materials and Methods*.

target inhibition, are taken to indicate that the interaction of CTL 8.4 with K7 and C1 occurs with lower "avidity" than with the $SB2^+$ cells.

Effect of decreasing available antigen on the target cell. The "avidity" of an effector-target interaction is thought to result from the cumulative effect of many antigen-specific (and antigen-nonspecific) bonds between effector and target. If so, the "avidity" of the interaction could be reduced by adding to the mixture concentrations of anti-Ia antibodies that decrease the amount of available antigen on the target (43). Two anti-Ia antibodies were used: SG171, which is a broad anti-Ia monoclonal antibody reactive with DR and SB (39, 44), and B7/21, which is selective for the SB/FA family of molecules (40, and unpublished observations). The concentrations of antibody chosen were ones that resulted in 30% or less inhibition of the lysis of clone 8.4 (Fig. 5, panel *A*). In the presence of anti-Ia antibody there were dramatic increases in the susceptibility of lysis to inhibition by anti-T3 and anti-T4 antibodies (Fig. 5, panels *B* and *C*). The effect of SG171 (anti-Ia) was most dramatic. In the presence of a concentration of SG171 that produced only 11% inhibition, the lysis became susceptible to inhibition by T3 at less than 5 μg/ml and to anti-T4 at less than 1 μg/ml (instead of being resistant to inhibition by either at 25 μg/ml). Similar effects were observed with B7/21 (anti-SB antibody); it is noteworthy that the higher concentration of B7/21 resulted in a greater inhibition of lysis by itself than did SG171 (30 vs 11%), and yet it resulted in less "potentiation" of anti-T3 and anti-T4 inhibition.

Variability in inhibition is characteristic of anti-T3 and anti-T4 but not anti-LFA-1. If anti-T3 and anti-T4 antibodies are both less able to block high "avidity" interactions, then perhaps all antibodies that block CML will also be less able to block high "avidity" interactions. Other antibodies known to inhibit CML have been included in many experiments to assess this possibility (anti-LFA-1, anti-LFA-2, anti-LFA-3, and anti-Ia); the least variation in susceptibility to inhibition has been seen for anti-LFA-1. Summary data are shown in Figure 6, which contrasts the consistency of anti-LFA-1 inhibition with the variability of anti-T3 and anti-T4 inhibition in three different experimental approaches. Figure 6, panel *A* compares inhibition of a high "avidity" clone (8, 4), two intermediate "avidity" clones (8.5 and 8.7), and a low "avidity" clone (8.10). Figure 6, panel *B* compares inhibition of the specific lysis of 8.4 on an SB2-positive target (B17) and two SB2-negative cross-reactive targets (K7 and PG2). Figure 6, panel *C* compares inhibition of lysis of the same clone (8.4) on a specific target in the absence or presence of limited amounts of anti-Ia antibody to reduce interaction "avidity". The data demonstrate wide variations in anti-T3 and anti-T4 inhibition, which correlates generally with differences in "avidity". Anti-T3 and anti-T4 inhibition generally parallel each other, but not exactly. Inhibition by anti-LFA-1 is largely invariant despite changes in "avidity"; however, some variation can be seen (e.g., Fig. 6, panel *C*). Anti-LFA-1 inhibition has been studied with another antibody (MHM23; 14) in the latter two approaches, and the finding or relative invariance of susceptibility was reproduced (data not shown).

DISCUSSION

The present study provides additional evidence that different effector-target interactions vary markedly in their susceptibility to inhibition by antibodies against T3 and T4, but indicates that inhibition by antibodies against the LFA-1 molecules does not vary markedly for these effectors. Furthermore, the variations are associated with differences in the "avidity" of the particular effector-target interaction. These findings are consistent for variations in "avidity" experimentally achieved in three different ways: different effectors on the same target, the same effector on specific and cross-reactive targets, and an effector assayed in the presence of sub-

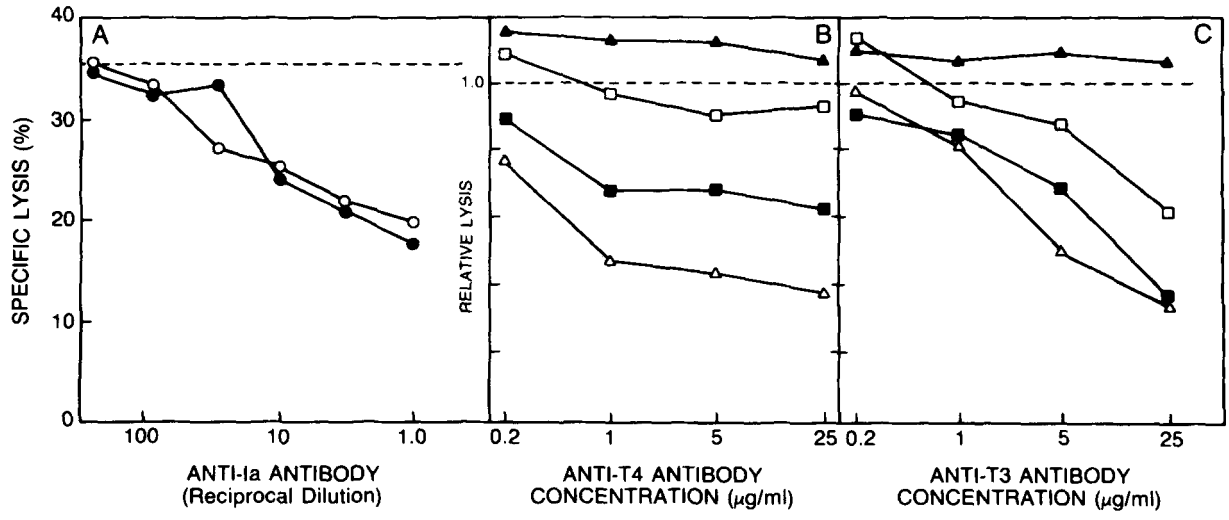

Figure 5. Enhancement of anti-T3 and anti-T4 antibody blocking by suboptimal concentrations of anti-Ia antibody. Titrations curves for blocking by anti-Ia antibody alone are shown in *panel A*: SG171 (a broad anti-Ia ○, titration started at concentraiton of 1/75 ascites) and B7/21 (SB specific ●, titration started at 1/3 culture supernatant); the *dashed line* represents the level of lysis in the absence of antibody. Inhibition by anti-T4 antibody (*panel B*) or anti-T3 antibody (*panel C*) was assayed in the presence (or absence) of low concentrations of those anti-Ia antibodies. The results shown indicate the inhibitory effect of anti-T3 (or anti-T4) per se, because they are shown relative to lysis in the presence of anti-Ia alone. For titrations in the absence of anti-Ia antibody (▲), the control value was 41% specific lysis. For titrations in the presence of SG171 at 1/750 ascites (△), the control value was 37% (11% inhibition). For titrations in the presence of B7/21 (1/18 culture supernatant ■ or 1/90 culture supernatant □), the control values were 29% lysis (30% inhibition) and 35% lysis (16% inhibition). The *dashed lines* in *panels B* and *C* represent lysis in the control condition (i.e., with anti-Ia but no anti-T3 or -T4).

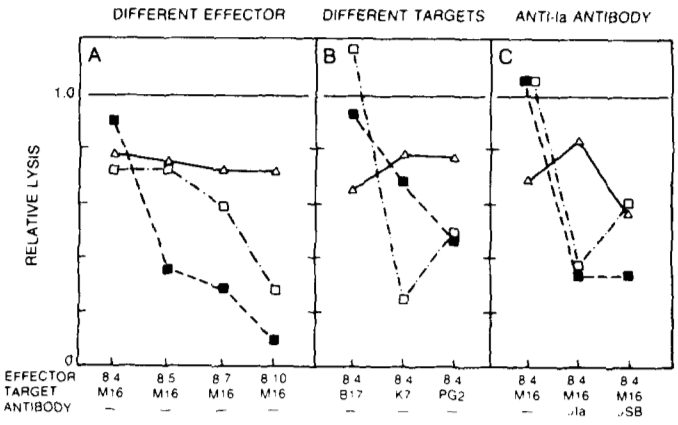

Figure 6. Overall comparison of anti-T3 and anti-T4 antibody blocking with anti-LFA-1 antibody blocking. High and low "avidity" interactions between effector and target were achieved in each of three different ways: different effectors on the same target (*panel A*), the same effector on different targets (*panel B*), and a single effector population assayed in the presence or absence of suboptimal concentrations of anti-Ia antibody (*panel C*). For each effector-target-Ia antibody combination, the results have been normalized to show lysis relative to lysis in the absence of anti-T3, -T4, and -LFA-1 antibodies. The *lines* connecting the *points* for anti-T3 (■), anti-T4 (□), and anti-LFA-1 (Δ) are to emphasize visually the variability for anti-T3 and anti-T4 inhibition and the relative constancy of anti-LFA-1 inhibition.

optimal concentrations of anti-Ia antibodies.

Previous studies have demonstrated variation between different clones (assayed on the same target) in their susceptibility to blocking by anti-T4 antibodies (1, 31); this correlated inversely with the "avidity" of the clone's interaction with that target, as estimated by the resistance to dissociation of their functional CTL-target cell conjugates (1). Complementary findings have been reported for the murine T4 analog, L3T4. Marrack *et al.* (43) reported that a panel of ovalbumin-specific L3T4$^+$ T cell hybridomas was very heterogeneous with respect to the amount of anti-L3T4 required to inhibit production of interleukin 2 (IL 2) induced by specific antigen plus the MHC. Those T cell hybrids that were most resistant to anti-L3T4 blocking responded best to low doses of antigen, and were the most difficult to block with anti-Ia antibodies. Furthermore, the susceptibility of the resistant hybrids to anti-L3T4 inhibition could be enhanced by the addition of low amounts of anti-Ia antibody, as was observed in the present study. The inference generally drawn from all of these studies has been that the T4 molecule is an accessory molecule that interacts with monomorphic Ia determinants but is necessary only to facilitate interactions that are of weak "avidity". These hypotheses for the T4 molecule are homologous to those that have been proposed for the function of the Lyt-2 molecule on Lyt-2$^+$ murine cells (35, 45, 46).

The results of the present study also demonstrate variations between different effector-target interactions in their susceptibility to inhibition by anti-T3 antibody, as has been suggested previously (1, 31). Our findings (1) differ from those of Moretta and co-workers (31), who found no correlation between inhibition by anti-T4 (or anti-T8) antibody and anti-T3 antibody. Our data indicate a statistically significant ($p = 0.03$) correlation between those two parameters, both of which also correlate with clonal "avidity" (1; and Fig. 1). Furthermore, Moretta et al. (31) describe CTL clones that cannot be inhibited by anti-T3 antibody under the conditions tested: blocking with anti-T3 antibody in solution or after modulation with anti-T3 antibody treatment; they inferred that activation of some clones could occur independently of the T3 structure. One of our clones, 8.4, is resistant to anti-T3 inhibition at concentrations that inhibit cytotoxicity by the other clones tested (≤25 μg/ml antibody); however, lysis by this clone can be inhibited by much higher concentrations of anti-T3 antibody (R. Hoffman, unpublished observations) when assayed on a cross-reactive target (Fig. 4) or when assayed in the presence of limiting amounts of anti-Ia antibody (Fig. 5). We interpret these results to indicate that a very small number of available T3 molecules is sufficient for the activation of some clones.

The present study shows remarkable consistency in the potency of anti-LFA-1 inhibition of different effector-target interactions. This finding of invariant inhibition is an essential internal control in these studies to show that the blocking efficiencies of all anti-effector antibodies do not vary in the manner observed for anti-T3 and anti-T4. Without that control, a plausible argument could be made that higher "avidity" interactions would be more difficult to block by any antibody. Our findings are consistent with those of MacDonald and co-workers (45), who found that murine cytotoxic clones that differ in susceptibility to anti-Lyt-2 inhibition are not markedly different in susceptibility to anti-LFA-1. However, invariability of anti-LFA-1 inhibition is not observed in all experimental systems. Golde et al.[2] observed variations in anti-LFA-1 inhibition of IL 2 secretion by murine T cell hybridomas that generally correlated with inhibition by anti-T4, i.e., low affinity clones were generally most susceptible to inhibition. Springer and co-workers (47) found that secondary CTL effectors (presumably of higher affinity) were harder to inhibit than primary effectors. Lectin-dependent cellular cytotoxicity can be inhibited by anti-LFA-1 at low lectin concentrations but not at high concentrations (47). It is unclear why anti-LFA-1 shows relatively invariant inhibition of CTL recognition by our clones and those of MacDonald (45), but not of other T cell recognition systems. Perhaps different T cell interactions differ in their dependence on the LFA-1 molecule in ways not related to affinity.

With respect to variations of efficiency of blocking, anti-T3 and anti-T4 inhibition are more similar to each other than to anti-LFA-1 inhibition. This is somewhat surprising inasmuch as anti-T4 and anti-LFA-1 inhibit at the phase of conjugate formation, whereas anti-T3 inhibits subsequent to that. Anti-LFA-1 blocks visible conjugate formation (3). Anti-T4 blocks formation of functional conjugates (2) and is able to partially disrupt previously formed functional conjugates (1). In contrast, anti-T3 does not appear to block conjugate formation but blocks at the post-recognition phase (17, 18). Thus, of two antibody that inhibit conjugate formation, only one shows striking variations in efficiency of blocking different effector-target interactions. This suggests that the mechanisms by which anti-LFA-1 and anti-T4 antibodies produce inhibition of cytotoxicity are different. Anti-LFA-1 antibody may simply sterically hinder the binding of the LFA-1 molecule to its ligand on the target cells. Anti-T4 antibody may block cytotoxicity by disrupting the formation of a "T cell molecular recognition complex" composed of T4, and T3 complex, and the antigen-specific receptor. Although Ti and T3 have been shown to

be associated physically with each other by co-precipitation (33, 48) and co-modulation (28, 48), T4 has not been found to share that association. However, inclusion of T4 in such a complex might only occur in the presence of antigen. Variations in susceptibility to anti-T4 as well as anti-T3 blocking could result from variations in the ability of clones to form such recognition complexes.

The finding of "avidity" dependence of anti-T3 antibody blocking may seem paradoxical at first, because the T3 molecule is thought to be involved in triggering but not in the interaction of antigen with receptor. However, consideration of the events involved in the interaction indicates that not only conjugate formation but also triggering could be highly affinity dependent. Cytotoxicity proceeds by formation of a conjugate with the target than triggering for delivery of the lethal hit. Clonal variations in affinity of the antigen-specific receptor (Ti) for its antigen will affect both phases of the interaction. A high affinity Ti-antigen interaction will result in the rapid formation of many Ti-antigen bonds between effector and target. (This is particularly true if both Ti and antigen are mobile in the membrane and can diffuse into the area of contact, as is probably the case for Ti and the specific antigen in this case, SB2.) A high affinity Ti-antigen interaction will result in the formation of more bonds and a stronger interaction, possibly a larger area of contact, and possibly a longer period of interaction. Those advantages of conjugate formation by a high affinity clone may make it relatively independent of the binding by some accessory molecules such as T4 (but not LFA-1). Inhibition of triggering might also be expected to be strongly affinity dependent. As outlined above, the number of Ti-antigen bonds formed will be dependent on affinity. Detailed discussion of inhibition by anti-T3 antibody is complex because the mode of triggering is not known and the mode of inhibition by anti-T3 is also not known. The simplest model of triggering would be that antigen binding per se generates a signal, and that triggering requires some threshold number of such signals. In this model, anti-T3 blocking of high affinity interactions would be more difficult than low affinity, because the blocking would have to interfere with the signal from many more antigen-Ti interactions. An alternate model for triggering is that clustering of receptors generates the signal that triggers the cytotoxic reaction; such models have been postulated, for example, for IgE-induced mast cell degranulation (49), and are attractive for T cell activation particularly in light of the evidence that anti-T3 induced mitogenesis depends on cross-linking (26). Such models predict that triggering will be highly dependent on the local concentration of Ti-antigen bonds (even more so than the first model), and therefore predict marked affinity-dependent variations in the efficiency of anti-T3 inhibition.

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