THE ROLE OF L3T4 IN RECOGNITION OF Ia BY A CYTOTOXIC, H-2D^d-SPECIFIC T CELL HYBRIDOMA

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Most T cells recognize antigen in association with either major histocompatibility complex (MHC)[1] class I or II molecules. This has led to the hypothesis that the specificity of the T cell receptor is for self MHC and foreign antigen. Recently, it has been shown that the expression of T cell surface markers, T4/T8 in man (1–10) and L3T4/Lyt-2 in the mouse (11–19), is generally associated with the T cells' MHC class restriction, rather than their functional subpopulations as previously thought. In general, T4 or L3T4 antibodies block the function of class II-restricted T cells (1–9, 16–19) whereas T8 or Lyt-2 antibodies functionally block class I-restricted T cells (1, 2, 5, 7–9, 11–15).

These T cell surface markers may interact with nonpolymorphic determinants of the MHC molecule since these T cell surface markers are not polymorphic, as opposed to the T cell antigen receptor, which is polymorphic and recognizes antigen in association with a polymorphic determinant of the MHC. Perhaps a combination of at least these two interactions leads to a cumulative binding avidity high enough to trigger T cell function. This hypothesis was recently tested (19) by using a large panel of antigen (Ag) and I region (Ag/I)-restricted murine T cell hybridomas. The ability of the panel of hybrids to respond to low concentrations of antigen was correlated with insensitivity to anti-Ia or anti-L3T4 blocking. These results suggest that a T cell with an Ag/I receptor with a high avidity for Ag/I does not require an L3T4/I interaction and, conversely, that T cells with low avidity for Ag/I require the L3T4/I interaction for triggering. This implies that L3T4 may be important in cell-cell interaction but not for antigen/MHC-restricted recognition.

A novel T cell hybridoma (20) with antigen specificity for a class I MHC antigen allowed us to test directly the role of L3T4. The hybridoma, 3DT52.5,

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[1] *Abbreviations used in this paper*: Ag, antigen; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; IL-2, interleukin 2; MHC, major histocompatibility complex; SMEM, Eagle's minimum essential medium supplemented as described; SN, supernatant.

TABLE I
Monoclonal Antibodies Used in These Experiments

Monoclonal antibody	Target molecules	Source	Reference
GK 1.5	L3T4	SN	16
KJ12-98.5	Ag receptor on T cell hybridoma 3DT52.5	Ascites	26
M12-4	Lyt-1	SN	27
53.7	Lyt-2	SN	28
M5/114	$I\text{-}A^{d,b}$, $I\text{-}E^{d,k}$	SN	29
10.2.16	$I\text{-}A^{k,s,f,r,u}$	SN	30
T24/31.7	Thy-1	SN	31
28.14.8	L^d(C2)	SN	32
30.5.7	L^d(N,C1)	SN	32
34.2.12	D^d(C2)	SN	33
34.5.8	D^d(N,C1)	SN	33

expresses L3T4 and recognizes a D region gene product of the H-2^d haplotype (19). The exact nature of the antigen recognition was unclear since 3DT52.5 did not respond to all D^d stimulators used.

In this paper, we have examined the fine specificity of recognition by 3DT52.5. Using L cells transfected (21–24) with either the D^d gene or recombinant genes, where the exons encoding the N and C1 domains of D^d and L^d have been exchanged, as stimulators we found that 3DT52.5 recognizes the N/C1 domain of D^d. Moreover, 3DT52.5, which produces interleukin 2 (IL-2) after activation, was found to lyse D^d target cells. We have used both functions of this T cell hybridoma to investigate the role of L3T4 in recognition of D^d using Ia^+ and Ia^- cells to present D^d.

Materials and Methods

Mice. C57BL/6, BALB/cBy, A/J, AKR, and (BALB/c × DBA/2)F_1 mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10A.5R/SgSn mice were bred in our animal facility from breeding pairs originally from The Jackson Laboratory.

Cell Lines. P815 (H-2^d), MBL2 (H-2^b), and RDM4 (H-2^k) were maintained by weekly passage in ascites of (BALB/c × DBA/2)F_1, C57BL/6, and AKR mice respectively. A20.2J.AG (A20)(H-2^d, Ia^+) was maintained in vitro.

L cells ($Lmtk^-$, DAP-3) (21) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, glutamine, and antibiotics in a humidified 5% CO_2/95% air environment. Cloned L cells that were transfected with H-$2L^d$ (T1.1.1), H-$2D^d$ (T4.8.3) genes (21, 23) and with recombinant genes in which the exons encoding the N and C1 domains of H-$2D^d$ and H-$2L^d$ had been exchanged (22, 24), indicated as H-$2L^d/D^d$ (T37.1.3) and H-$2D^d/L^d$ (T37.2.1) (see Results), were maintained in hypoxanthine, aminopterin, thymidine (HAT)-supplemented media. Cells were detached by versene (Gibco Laboratories, Grand Island, NY) treatment.

Monoclonal Antibodies. Monoclonal antibodies were prepared, either as a cell-free supernatant of hybridoma cultures or as an ascitic fluid from irradiated mice carrying the hybridoma as a tumor. A list of monoclonal antibodies and their sources used in these experiments is shown in Table I. The concentration of the various antibodies varied according to experimental protocols, as indicated in Results. Antibodies were used at saturating concentrations.

T Cell Hybridomas. The T cell hybridomas used in these experiments have been

previously described (20, 34). Briefly, 3DT52.5 resulted from a fusion of (T,G)-A-L–primed T cell blasts with an azaguanine resistant subline of the AKR thymoma BW5147. 3DT52.5 was characterized as a hybridoma that secretes IL-2 in response to a class I antigen coded for the H-2D^d region, although the molecular nature of the antigen is unknown. A variant of 3DT52.5 was prepared by passaging the cloned hybridoma in tissue culture and recloning the hybrids at limiting dilution. 3DT52.5.8 was characterized as an IL-2-producing hybridoma with the same antigen specificity as the parent, but which did not express the L3T4 marker.

Culture Conditions. All cell lines were grown and tested in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin (100 μg/ml), and streptomycin (100 μg/ml). For stimulation of IL-2 secretion, 1×10^5 hybridoma cells were co-cultured with various numbers of spleen cells or tumor cells (previously exposed to 10,000 rad gamma irradiation and chosen to result in suboptimal stimulation so that blocking could be detected) in a total volume of 1 ml. After 20–24 h, supernatants (SN) were removed and assayed for the presence of IL-2.

IL-2 Assay. SN were titrated by serial twofold dilutions and assayed for IL-2 by their ability to support the proliferation of the IL-2-dependent T cell line, HT-2 (35). Proliferation was assessed by the incorporation of [^{3}H]thymidine during a 4-h pulse with 1 μCi [^{3}H]thymidine/well. Data are presented as U/ml relative to the [^{3}H]thymidine incorporation of HT-2 from a standard rat concanavalin A (Con A) SN. The half-maximal incorporation from the Con A SN titration was defined as 100 U/ml IL-2. The standard error is derived from the mean of the cpm from the HT2 assay. Each table represents a series of at least three experiments.

Cytotoxic T Lymphocyte Assay. Target cells were prepared by harvesting tumor cells and incubating ~5×10^6 cells with 150 μCi $Na_2\,^{51}CrO_4$ (New England Nuclear, Boston, MA) for 1 h at 37°C in Eagle's minimum essential media supplemented with 10% fetal bovine serum, penicillin, and streptomycin, 1% nonessential amino acids, and 2 mM L-glutamine (SMEM). Labeled cells were washed three times and 5×10^3 cells were mixed with various numbers of hybridoma cells in 150 μl of SMEM in V-bottomed microtiter plates (Linbro Chemical Co., Hamden, CT). Plates were spun (100 *g*, 5 min) and incubated for 6 h at 37°C. Plates were spun again and 100 μl of SN harvested to determine the amount of radioactivity released. Specific lysis was calculated as follows: 100 × [(^{51}Cr released by hybridomas + target cells) − (^{51}Cr released by target cells alone)/(maximal ^{51}Cr released with 1.5% Triton X-100) − (^{51}Cr released by target cells alone)]. Standard deviation of replicates was <5%. The experiments presented are representative of at least three experiments.

Enzyme-linked Immunosorbent Assay (ELISA). Binding of antibodies to T cell hybridomas was assessed with an ELISA, as previously described (20). 5×10^5 hybridoma cells were incubated in media with a test antibody in 96-well culture dishes that were precoated with fetal calf serum (FCS). After a 1-h incubation at 0°C, the plates were washed with phosphate-buffered saline-2% FCS. This was followed by a 1-h incubation with 100 μl of a 1:250 dilution of alkaline phosphate-coupled rabbit anti-mouse Ig antibody. After thorough washing, 175 μl of substrate, para-nitrophenyl phosphate as a 1.67 mg/ml solution in 0.1 M glycine buffer, pH 10.4 with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$, was added. The plates were incubated for 1 h at 37°C, then read for optical density (OD) with an ELISA reader using a 405 nm filter. The results are presented in arbitrary units of OD after subtraction of the background OD of wells lacking the test antibody.

Results

Mapping of the Fine Specificity of 3DT52.5. Previous work (20) to determine the specificity of 3DT52.5 showed that this T cell hybridoma was specific for a gene product of the D region of the MHC. Not all H-2^d haplotype, D^d-bearing cells were effective stimulators; therefore, the exact specificity of 3DT52.5 was unknown. To test directly whether 3DT52.5 is specific for D^d or another H-2 antigen that is not serologically defined but genetically linked to D^d, L cells

TABLE II
3DT52.5 Responds to the N/C1 Domain of D^d

	3DT52.5 stimulated with:	IL-2 production
		U/ml
Exp. 1	BALB/c spleen ($H\text{-}2^d$)	330 ± 13
	P815 ($H\text{-}2^d$)	478 ± 8
	MBL2 ($H\text{-}2^b$)	9 ± 1
	DAP ($H\text{-}2^k$)	<5
	T1.1.1 (L^d)	<5
	T4.8.3 (D^d)	178 ± 20
Exp. 2	T37.1.3 (L^d/D^d)	16 ± 2
	T37.2.1 (D^d/L^d)	280 ± 13

10^5 3DT52.5 were cultured for 20 h with 5×10^5 BALB/c spleen cells, 10^5 tumor cells, or 10^5 L cells, as described in Materials and Methods.

transfected with the D^d or L^d genes were used as stimulators. The results of these experiments are shown in Table II. 3DT52.5 responded by producing IL-2 when stimulated with BALB/c splenocytes, P815, or L cells transfected with the D^d gene but not with L cells transfected with the L^d gene. This demonstrates that 3DT52.5 is specific for the D^d gene product.

L cells that have been transfected with recombinant genes made by exchanging exons encoding various domains of L^d and D^d (22, 24) were used to determine the fine specificity of 3DT52.5. T37.1.3 is an L cell transfected with a recombinant gene and expresses a hybrid H-2 molecule composed of the N and C1 domains of L^d and the C2 domain of D^d; T37.2.1 is an L cell transfected with a recombinant gene composed of the exons encoding the N and C1 domains of D^d and the C2 domain of L^d. As shown in Table II, experiment 2, only T37.2.1 stimulated IL-2 production by 3DT52.5, demonstrating that 3DT52.5 is specific for the N and/or C1 (N/C1) domains of the D^d molecule.

These results are confirmed by studies in which monoclonal antibodies were used to block stimulation of 3DT52.5 by the D^d-transfected L cell (shown in Table III). These experiments demonstrated that 3DT52.5 stimulation was blocked by antibody directed at the N/C1 domain of D^d and by a clone-specific anti-3DT52.5 D^d receptor antibody (KJ12-98.15) (25). Interestingly, antibody directed at the C2 domain of D^d appears to enhance IL-2 production by 3DT52.5. Collectively, these experiments map the recognition of 3DT52.5 to the N/C1 domain of the D^d gene product.

Lytic Activity of 3DT52.5. The unusual nature of the recognition of 3DT52.5, in that it directly recognizes a "self" class I molecule, led us to postulate that IL-2 production may not be its only function. The traditional association of cytotoxic T cell function with MHC class I recognition led us to assess whether 3DT52.5 also had cytolytic activity. 3DT52.5 was tested for cytotoxicity against a panel of targets, as shown in Fig. 1. 3DT52.5 lysed A20 and P815, both of which express D^d, but did not lyse RDM4, which expresses $H\text{-}2^k$. Therefore, 3DT52.5 has cytolytic activity with specificity for $H\text{-}2^d$. However, 3DT52.5 had no cytolytic activity against BALB/c blasts, YAC-1, or the transfected L cells (data not shown). Inability to lyse these targets may reflect quantitative differences in expression

TABLE III

3DT52.5 Is Inhibited by Antibody to the Idiotype of the T Cell Receptor and to the N/C1 Domain of D^d

	Antibody added directed against:*	IL-2 production
		U/ml
3DT52.5 stimulated with T4.8.3	—	872 ± 7
	3DT52.5 D^d receptor	219 ± 7
	Lyt-2	725 ± 3
	D^d(N,C1)	330 ± 6
	D^d(C2)	2,728 ± 81
	L^d(N,C1)	1,248 ± 83
	L^d(C2)	786 ± 40

3DT52.5 stimulated as in Table II, except at 10^6 cells/ml.
* Antibodies used as listed in Table I; final concentration of monoclonal Ab as SN was 20% and as ascites, 5%.

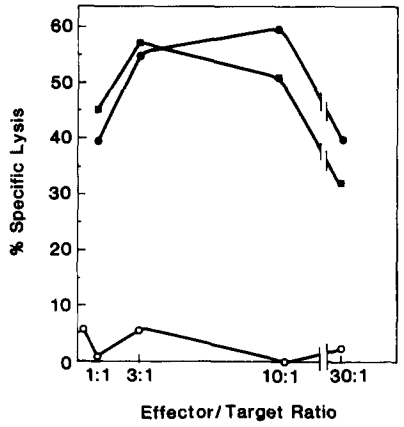

FIGURE 1. Cytolytic activity of 3DT52.5. 3DT52.5 killing of P815 (■), A20 (●), and RDM4 (○). Spontaneous release was 10–25% of total counts in a 6-h assay.

of D^d, differences in posttranscriptional modification of D^d, or differential expression of invarient target antigens. Perhaps the cytolytic event requires a higher avidity interaction with D^d than the interaction leading to IL-2 production.

Target and Effector Surface Antigens Important in 3DT52.5 Recognition. Recognition of the D^d molecule by 3DT52.5 resulted in both IL-2 production and cytolytic activity. The direct recognition of a class I molecule by an Lyt-1^+2^-,L3T4^+ T cell hybridoma (Table IV) affords the opportunity to study the functional recognition of Ia by L3T4. We compared the ability of anti-Ia and anti-L3T4 to block lytic activity (Fig. 2) and IL-2 production (Tables V and VI). The results, shown in Fig. 2, demonstrate that the cytolytic activity of 3DT52.5 was blocked by the addition of saturating concentrations of anti-Ia^d and by anti-L3T4 only when the target cell expressed Ia (shown by the A20 target, Fig. 2*B*). In contrast, the lysis of the Ia^- target, P815 (Fig. 2*A*), was unaffected by anti-Ia and anti-L3T4; the slight inhibition by anti-L3T4 was only seen in one experi-

TABLE IV

Expression of T Cell Surface Markers by T Cell Hybridomas

Antibodies directed against:	OD ± SE (×10³) on T cell hybridomas*	
	3DT52.5	3DT52.5.8
Thy-1	581 ± 30	620 ± 27
L3T4	164 ± 15	2 ± 3
Lyt-2	10 ± 8	2 ± 8
D^d receptor on 3DT52.5	353 ± 26	813 ± 35

* As described in Materials and Methods.

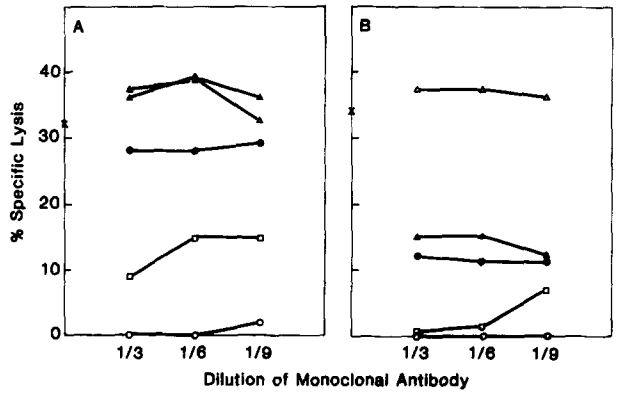

FIGURE 2. 3DT52.5 cytolytic T lymphocyte activity is blocked by anti-L3T4 and anti-Ia only when the target expresses Ia. Lytic activity of 3DT52.5 against P815 (*A*) and A20 (*B*). Antibodies used (as listed in Table I) were: no antibody (×); anti-IA^d IE^d (▲); anti-IA^k (△); anti-D^d N/C1 (□); anti-L3T4 (●); anti-D^d receptor on 3DT52.5 (○). The anti-D^d receptor antibody containing ascites was diluted 10-fold more than other antibodies used. Effector/target ratio was 10:1. The SE of the percent specific lysis was <3%.

TABLE V

Stimulation of 3DT52.5 Requires the L3T4 Receptor When the Stimulator Is Ia^+

Antibody added to culture directed against:*	IL-2 production‡	
	3DT52.5 stimulated with:	
	T4.8.3	A20
—	100	100
D^d receptor on 3DT52.5	<5	9
L3T4	104	29
Lyt-1	100	98

* Antibody added to cultures were 20% for SN and 5% for ascites.

‡ 10^5 3DT52.5 were cultured for 20 h with 5×10^4 T4.8.3 or A20 cells as stimulators. The amount of IL-2 production was determined by HT-2 assay, and is presented as the percent of IL-2 production in the absence of added antibody. Amount of IL-2 produced: 480 U/ml with T4.8.3 and 580 U/ml with A20. The SE from the HT-2 assay was ≤10% of the total cpm.

TABLE VI

L3T4 Receptor Recognizes a Nonpolymorphic Determinant of Ia

Antibody added directed against:	IL-2 production*‡			
	3DT52.5 stimulated with:			
	Exp. 1		Exp. 2	Exp. 3
	T4.8.3 D^d	A20 D^dIa^d	B10.A(5R) $D^dIA^bIE^k$	A/J D^dIa^k
—	100	100	100	100
$I\text{-}A^{d,b}$, $I\text{-}E^{d,k}$	106	49	57	91
$I\text{-}A^k$	100	119	100	31
D^d(N/C1)	10	22	27	14
L3T4	ND§	ND	33	8

* 10^5 3DT52.5 were stimulated for 20 h with 5×10^4 T4.8.3 or A20 or 5×10^5 A/J splenocytes. IL-2 production was calculated as in Table V. Amount of IL-2 produced: 50 U/ml with T4.8.3, 148 U/ml with A20, 192 U/ml with A/J spleen, and 120 U/ml with B10.A(5R).

‡ See Table IV.

§ Not done.

ment. The lytic activity of 3DT52.5 was completely blocked by anti-D^d and anti-D^d receptor on 3DT52.5 on both the Ia^+ and Ia^- targets. Comparable results were seen when IL-2 production was assayed (Table V and Table VI, experiment 1). These experiments suggest that while 3DT52.5 has specific receptors for the D^d molecule, recognition of Ia determinants via the L3T4 molecule may influence the antigen-dependent triggering of 3DT52.5 T cell function.

To determine whether L3T4 reacts with a polymorphic determinant of Ia, the effect of anti-Ia on the stimulation of 3DT52.5 with A/J (D^d,Ia^k) and B10.A(5R)(D^d,IA^b,IE^k) splenocytes was studied. The results, shown in Table VI, demonstrate that anti-L3T4 and the appropriate anti-Ia blocked the interaction of 3DT52.5. The correlation of anti-L3T4 and anti-Ia blocking is highly specific, as shown below. This suggests that L3T4 interacts with a nonpolymorphic determinant of Ia. Although 3DT52.5 has specificity for the D^d antigen alone, the presence of Ia on the stimulator cell links the triggering event of D^d recognition to the L3T4/Ia interaction.

Function of an L3T4$^-$ Variant of 3DT52.5. 3DT52.5.8 is a L3T4$^-$ variant of 3DT52.5 (Table IV). 3DT52.5.8 produced IL-2 in response to T4.8.3 and A20 (Table VII) but, in contrast to 3DT52.5, 3DT52.5.8 was not blocked by anti-L3T4 or anti-Ia reagents. This demonstrates that anti-Ia blocking can be correlated with the expression of L3T4 when the T cell receptor for antigen is not restricted by Ia. These results also show that the clonotypic 3DT52.5 antigen receptor is not restricted by Ia and that triggering of 3DT52.5 is not strictly related to an L3T4/Ia interaction. We cannot exclude the remote possibility that another cell surface structure is coordinately expressed with L3T4, blocked by steric hindrance, and also not expressed by 3DT52.5.8.

Surprisingly, 3DT52.5.8 did not demonstrate any lytic capacity (data not shown). However, this does not necessarily imply a requirement for L3T4 in cytolysis, since 3DT52.5.8, a spontaneous variant, may possibly have lost expression of other important functional molecules.

TABLE VII
An L3T4$^-$ Variant of 3DT52.5 Is Not Inhibited by Anti-Ia

Antibody added directed to:*	IL-2 production*	
	3DT52.5.8 stimulated with:	
	T4.8.3	A20
—	100	100
D^d (N/C1)	36	31
IA^d, IE^d	100	91
IA^k	120	79
L3T4	120	91

* As in Table V. Amount of IL-2 produced: 483 U/ml with T4.8.2 and 263 U/ml with A20. In the same experiment, 3DT52.5 stimulation with A20 was blocked by anti-Ia^d and anti-L3T4.

Discussion

Recently, there has been an accumulation of evidence suggesting that L3T4 is the murine homologue of the human T cell marker Leu3/T4 (16–20) and that these human and mouse T cell antigens are involved in the recognition of class II molecules (1–8, 16–19). Similar evidence leads to the association of Lyt-2 and Leu-2/T8 with the recognition of class I antigens (1, 2, 5, 7–9, 11–15). The interaction of T cell surface markers and MHC antigens may be involved in adding to the cumulative avidity needed to trigger T cell function (20). A direct test of this hypothesis has not been possible since it has been difficult to separate the binding of the T cell antigen receptor to an Ag/Ia complex from the L3T4 interaction with Ia. 3DT52.5 is a novel T cell hybridoma in that it has an antigen receptor specific for a self class I antigen, is Lyt-2$^-$, and expresses L3T4 (20, 21). 3DT52.5 was shown to be antigen specific for the D^d molecule by its ability to produce IL-2 when stimulated with L cells transfected with the D^d gene. The fine specificity of its receptor is for the N/C1 domain of the D^d molecule, as shown by stimulation with L cells transfected with recombinant genes, in which the N/C1 domains of D^d and L^d were exchanged, as well as by antibody blocking of IL-2 production.

The experiments presented in this paper allowed the separate evaluation of the T cell receptor recognition of antigen and the role of L3T4. The recognition of D^d by an Lyt-2$^-$, L3T4$^+$ T cell hybridoma gave us the ability to separately evaluate the interaction of L3T4 with class II antigens. We have shown that 3DT52.5 is inhibited by anti-L3T4 and anti-Ia only when the stimulator cell expressing D^d is also Ia^+. Although anti-L3T4 is bound to 3DT52.5 in both cases, the functional inhibition by anti-L3T4 occurs only when the stimulator is Ia^+. The anti-antigen receptor on 3DT52.5 and anti-D^d (N/C1 domains) completely inhibits the reactivity of 3DT52.5 to both Ia^+ and Ia^- cells. The ability to block T cell effector function of 3DT52.5 with anti-L3T4 implies that the L3T4/I interaction is involved in triggering by Ia^+ splenocytes or A20 but not with P815 or T4.8.3. The blocking by anti-L3T4 and anti-Ia on Ia^+ stimulator or target cells was always less than total blocking. This may imply that while the D^d/D^d receptor on 3DT52.5 interaction is essential for triggering effector cell function, the L3T4/I interaction may serve an accessory function. Since the

avidity of the recognition of D^d on these different cell types by the 3DT52.5 antigen receptor can not be quantitated, we can only suggest that the L3T4/I interaction may be responsible for increasing the interaction avidity with the Ia^+ cells to the triggering threshold. This may reflect the relative amount of expression of D^d on the target cells used, since T4.8.3 and P815 have more D^d than A20 (data not shown). Since A20 has less D^d, the L3T4/I interaction may raise the cumulative binding avidity to the triggering threshold. 3DT52.5.8 may bypass this requirement when stimulated with A20 by the increased expression of the D^d receptor (Table IV).

The experiments presented demonstrate that while 3DT52.5 has antigen specificity for D^d, recognition of Ia by the L3T4 molecule can affect the triggering of effector T cell function. When the avidity of the T cell antigen receptor for antigen is less than the triggering threshold, the L3T4/I interaction may raise the cumulative binding avidity to the threshold. If the avidity of the T cell receptor for its antigen is high, there may be no additional requirement for other interaction molecules. Thus, one can postulate an ordered requirement for cell-cell interaction, starting with the T cell antigen receptor, adding the L3T4/I or Lyt-2/class I interactions and other interaction antigens such as LFA-1, LFA-2, and LFA-3 (36) (LFA-2 and LFA-3 have as yet only been defined in human T cell interactions), all leading to effective T cell triggering. Another possible explanation for the inhibition seen with anti-L3T4 and anti-Ia may be that the interaction of Ia, L3T4, and anti-L3T4 decreases the membrane mobility of 3DT52.5 or the D^d-presenting cell, thus interfering with a necessary activation signal. This type of effect has been suggested by the decrease in B cell membrane mobility after Con A binding to surface immunoglobulin (37).

L3T4 interacts with Ia, as shown by the ability of anti-L3T4 to block stimulation of 3DT52.5 by A/J and B10.A(5R) splenocytes and A20 tumor cells. These results demonstrate that the L3T4 receptor on 3DT52.5 can interact with Ia from three distinct haplotypes. Therefore, the L3T4 surface marker on 3DT52.5 may recognize a nonpolymorphic determinant of Ia shared by these distinct haplotypes. Preliminary results (not shown) suggest that L3T4 interacts with I-A and I-E.

The ability of anti-L3T4 to block class II-associated functions of cloned T cells (16–18), T cell hybridomas (16, 18, 20), and bulk T cell cultures (17, 19) implicates L3T4 in the recognition of class II antigens. Similar conclusions have been drawn from experiments with Leu3 and T4 in human studies (1–9) and regarding class I recognition by Lyt-2 and Leu-2/T8 (1, 2, 5, 7–9, 11–15). The L3T4 interaction with Ia has been shown to contribute to antigen recognition by Ag/I-specific T cell hybridomas at the level of recognition and binding to antigen-presenting cells (18) and in cytolytic T cell clones before the lethal hit (16, 17). The data presented in this paper directly demonstrate the recognition of Ia by L3T4 in the absence of an I region-restricted antigen interaction. Additionally, we have shown that L3T4 interacts with a nonpolymorphic determinant of Ia. In this manner, the L3T4 molecule may serve an accessory function to the T cell antigen receptor. Binding to the antigen-presenting cell occurs via both receptors. A high avidity T cell antigen receptor interaction can trigger the

cell to respond; in contrast, a low avidity T cell receptor may also require the L3T4/Ia interaction to gain the threshold-triggering avidity.

The physiological role of nonpolymorphic L3T4-mediated interactions between T cells and antigen-presenting cells remains unclear. Interaction of L3T4 with Ia may allow T cells to home to and screen potential antigen-presenting cells. Alternatively, the L3T4/I interaction may be involved in cell-cell communication.

Summary

The expression of T4/T8 surface markers on human T cells and of L3T4/Lyt-2 on murine T cells has lead to the association of these surface markers with recognition of either class II or class I major histocompatibility complex (MHC) antigens. It has been suggested that these T cell surface antigens interact with MHC antigens. We have examined the role of L3T4 in the recognition of D^d by the T cell hybridoma, 3DT52.5. This T cell hybridoma was found to be specific for the N/C1 domain of D^d. The recognition of a class I antigen by an Lyt-2^-, L3T4^+ T cell hybridoma allowed the separate evaluation of interactions between L3T4/Ia and the T cell antigen receptor, D^d. Recognition by this hybridoma resulted in the production of interleukin 2 (IL-2) and cytolytic activity. Antibody blocking experiments have demonstrated that L3T4 was involved in triggering the effector function of 3DT52.5 only on Ia^+ stimulator or target cells. We have demonstrated that an L3T4^+, D^d-specific T cell hybridoma, 3DT52.5, uses the L3T4 molecule to directly interact with nonpolymorphic Ia determinants.

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