Brief Definitive Report

CYTOTOXIC T LYMPHOCYTES INDUCED AGAINST ALLOGENEIC *I*-REGION DETERMINANTS REACT WITH Ia MOLECULES ON TRINITROPHENYL-CONJUGATED SYNGENEIC TARGET CELLS*

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The major histocompatibility complex codes for determinants which are recognized by and serve as targets for cytolytic T lymphocytes (CTL) (1). Antigens coded for by the *K* and *D* loci of the *H-2* complex can activate xenogeneic or allogeneic CTL (2, 3). In addition, the *H-2K* or *H-2D* gene products function as those molecules against which syngeneic CTL responses specific for chemical, viral, and minor H antigens are directed (4–8).

It has recently been shown that Ia determinants can also serve as target antigens for distinct but weaker CTL responses (9–13). Those clones which recognize Ia antigens see them independently of *K*- or *D*-coded antigens as shown in genetic studies and by antisera-blocking experiments (12, 13). We have proposed that the existence of clones of CTL specific for *I*-region-coded determinants is not fortuitous; rather these clones specifically recognize Ia determinants and may have an immunoregulatory role. These CTL may affect those immune functions which are at least partially dependent on or controlled by *I*-region-coded molecules.

Two predictions can be made and tested concerning the role of Ia determinants in cytolytic systems and the role, if any, of *I*-region-specific CTL in regulating the immune response: (*a*) that if as we and others have shown, certain Ia specificities can serve as a third series of major histocompatibility antigens, then Ia antigens should be susceptible to the same types of antigenic modification as *H-2K*- or *H-2D*-coded structures and thus serve as targets for CTL directed against modified-self in selected systems; and (*b*) that allogeneically induced *I*-region-specific CTL should demonstrate cross-reactivity with targets bearing modified syngeneic *I*-region-coded determinants. Data will be presented which demonstrates that trinitrophenyl (TNP)-modified syngeneic *I*-region determinants can serve as targets for CTL induced by allogeneic Ia antigens.

Materials and Methods

Mice. 6- to 12-wk-old male and female mice from our own breeding colony or purchased from The Jackson Laboratory, Bar Harbor, Maine, were used in these studies. Some A.TL mice were the gift of Dr. David Sachs, National Cancer Institute, Bethesda, Md. The B10.T(6R) and B10.AQR strains are referred to as 6R and AQR, respectively, throughout this communication.

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TABLE I
Ability of I-Region-Specific CTL to Lyse TNP-Modified Targets

Experiment	Responder	Stimulator	Target*	% Specific release of ^{51}Cr
1	AQR(*qkdd*)‡	6R(*qqqd*)	6R-TNP	64.3
	AQR	–	6R-TNP	12.0
	AQR	6R	AQR-TNP	40.0
	AQR	–	AQR-TNP	3.5
	AQR	6R	AQR	−0.3
2	A.TH(*sssd*)	A.TL(*skkd*)	A.TL	18.0
	A.TH	A.TL	A.TH	4.0
	A.TH	A.TL	A.TH-TNP	31.0
3	AQR	6R	6R	20.5
	AQR	6R	6R-TNP	21.8
	AQR	6R	AQR	1.2
	AQR	6R	AQR-TNP	15.2

* Effector to target ratio was 100:1. Spontaneous release of LPS blast targets ranged from 30 to 40%.
‡ Indicates *H-2* alleles for *K*, *I*, *S*, and *D* regions.

Antisera. Alloantisera were raised by six to eight intraperitoneal injections of lymphoid cells. Donor-recipient combinations used and the specificity of each serum are given in the legend to Table III.

Generation of Effector Cells and the ^{51}Cr-Release Assay. The method used to generate cytotoxic effector cells to allogeneic targets has been described elsewhere (14). TNP coupling of target cells was accomplished according to published techniques (15). The ^{51}Cr-release assay used in this study and the method of antisera inhibition have been previously described in detail (15). Lipopolysaccharide (LPS)-induced blast spleen cells used as targets in the ^{51}Cr-release assay were produced by incubating 4×10^6 spleen cells with 10 μg of LPS in 2 ml of medium consisting of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and glutamine. LPS blasts produced in this fashion were shown to contain approximately 80% Ia-positive cells by complement (C)-mediated cytotoxicity.

Percent specific release was calculated as: $[(E - C)/(FT - C)] \times 100\%$; where E is isotope release in tubes containing immune effectors plus targets, C is isotope release in tubes containing normal spleen cells and targets (spontaneous release), and FT is maximum isotope release in tubes containing targets after freeze-thawing three times.

Results

Ability of TNP-Modified Syngeneic Spleen Cells to Act as Targets for I-Region-Specific CTL. Results in Table I illustrate again that CTL induced in mixed lymphocyte culture where the responder and stimulator differ only at the *I* region of the *H-2* complex lyse targets bearing the stimulating allogeneic *I* region. These same CTL also lyse syngeneic targets which have been chemically modified by TNP but will not lyse nonmodified syngeneic targets. TNP modification of allogeneic *I*-region-bearing targets did not alter specific release in this assay. Effector cells which lyse TNP-modified syngeneic and allogeneic Ia-bearing targets are eliminated by anti-Thy 1.2 antiserum plus C (results not shown). Therefore, chemical modification of syngeneic cell surface antigens by TNP allows CTL directed specifically at *I*-region-coded alloantigens to lyse these cells.

The magnitude of cytolysis of modified syngeneic *I*-region-bearing targets is similar to that observed on cells expressing allogeneic Ia antigens used for immunization. In several experiments at the same effector to target ratio (100:1), the mean specific lysis of the modified syngeneic target was 89% (±14%)

TABLE II
Specificity of CTL Induced to Allogeneic I-Region Determinants

Experiment	Responder	Stimulator	Target*	Responder TNP-target *H-2* identities‡	% Specific release of ^{51}Cr
1	A.TH(*sssd*)§	A.TL(*skkd*)	A(*kkdd*)	–	68.6
	A.TH	A.TL	A.TH-TNP	K, I, D	53.8
	A.TH	A.TL	A.SW-TNP(*ssss*)	K, I	57.6
2	AQR(*qkdd*)	6R(*qqqd*)	AQR	–	1.2
	AQR	6R	6R	–	21.2
	AQR	6R	AQR-TNP	K, I, D	15.2
	AQR	6R	B10.BR-TNP(*kkkk*)	I	15.5

* Effector to target ratio was 100:1. Spontaneous release of LPS blast targets ranged from 35 to 42%.
‡ *H-2* specificities shared between responder and TNP-coupled targets, excluding those products of the *S* region.
§ Indicates *H-2* alleles for *K*, *I*, *S*, and *D* regions.

TABLE III
*Antiserum Blocking of Cytolysis by CTL Induced to Allogeneic I-Region Determinants**

Experiment	Responder	Stimulator	Target‡	Antiserum§		
				Control	α-I^k	α-D^d
1	AQR(*qkdd*)	6R(*qqqd*)	6R-TNP	21.5	17.5	21.2
	AQR	6R	AQR-TNP	15.2	3.4	14.0
	AQR	6R	B10.BR-TNP(*kkkk*)	15.4	2.2	11.5
2	AQR	6R	6R	28.0	–	–
	AQR	6R	B10.BR-TNP	23.2	−0.2	15.5
	B6	6R	6R	64.2	66.4	37.8

* Values represent percent specific release of ^{51}Cr.
‡ Effector to target ratio was 100:1. Spontaneous release of LPS blast targets ranged from 30 to 40%.
§ Control consisted of SMEM-10; anti-Ia^k antiserum is A.TH anti-A.TL with a lytic titer of 1/1,280; anti-H-$2D^d$ is (B10 × LP.RIII) F_1 anti-18R with a lytic titer of >1/640. Both antisera were used at a final dilution of 1:12.
Indicates *H-2* alleles for *K*, *I*, *S*, and *D* regions.

of the lysis on the target bearing the stimulating allogeneic *I*-region determinants. Thus, under these conditions, clones of CTL stimulated by *I*-region differences may recognize TNP-modified syngeneic determinants as well as allogeneic Ia antigens on the presented target cells.

Table I further illustrates that culturing responder AQR spleen cells for 5 days without stimulator cells does not produce effectors which significantly lyse either allogeneic or modified syngeneic *I*-region-bearing targets. *I*-region-specific CTL also fail to lyse TNP-modified ACI rat targets (results not shown).

Specificity and Lack of H-2K or H-2D Restriction of the Cytolysis of Modified Syngeneic Targets by I-Region-Specific CTL. CTL induced by allogeneic Ia antigens show specificity in target lysis for Ia antigens and are not genetically restricted by the *K* or *D* loci of the *H-2* complex in effecting this lysis. Table II shows that CTL induced to allogeneic *I*-region differences lyse TNP-modified targets syngeneic at the *I* region irrespective of the alleles present at the *K* or *D* loci. This suggests that this lytic activity is not directed at modified *K*- or *D*-coded determinants, but at *I*-region-coded determinants and furthermore, that the cytolysis is not restricted by products of the *K* or *D* loci.

Table III demonstrates the specificity of modified syngeneic *I*-region cytolysis by antisera blocking. An antiserum specific for *I*-region-coded products on the modified AQR targets blocks the lysis of these targets by AQR *I*-region-specific

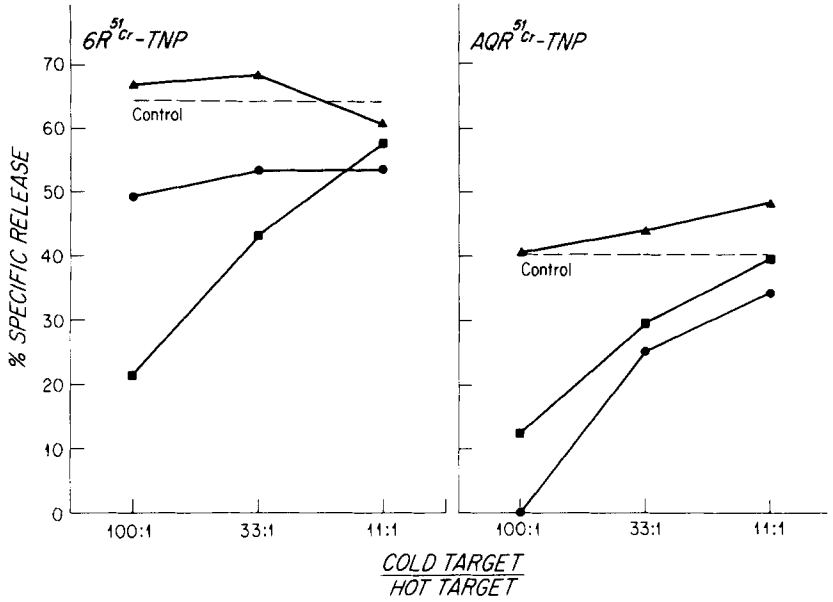

FIG. 1. Cold target inhibition of *I*-region-specific CTL. AQR anti-6R effectors were assayed on 6R-TNP (left panel) and AQR-TNP (right panel) ^{51}Cr-labeled targets. Nonradiolabeled AQR (▲ – ▲), 6R (■ – ■), and AQR-TNP (● – ●) spleen cells were used as cold target inhibitors at three cold to hot target ratios (100:1, 33:1, and 11:1). Control is lysis without cold targets added. Spontaneous release of LPS-blast targets was 40% for 6R-TNP and 38% for AQR-TNP.

CTL. In addition, this serum inhibits the lysis of B10.BR-TNP targets which share the *I-A* subregion with the AQR responder but differ at the *K* and *D* loci. This same anti-Ia reagent, while significantly inhibiting the lysis of targets bearing modified syngeneic *I*-region-coded determinants, does not reduce the lysis of targets bearing allogeneic Ia antigens used for stimulation.

An antiserum directed to the products of the $H\text{-}2D^d$ locus has no effect on the lysis of either allogeneic or modified syngeneic targets (bearing *d* alleles at the *D* locus) by CTL induced by allogeneic Ia antigens. This reagent does significantly reduce the lysis of 6R targets by allogeneic C57BL/6 (B6) CTL which are primarily directed at products of the $H\text{-}2K^q$ and $H\text{-}2D^d$ loci.

Thus, both in genetic studies and in antisera-blocking experiments, it can be shown that CTL induced by allogeneic Ia determinants will specifically lyse targets presenting TNP-modified syngeneic *I*-region-coded determinants. Clones of CTL induced by allogeneic Ia antigens when presented with targets bearing modified syngeneic *K*-, *I*-, and *D*-region-coded structures appear to recognize only the modified Ia determinants, indicating that these CTL see modified syngeneic Ia antigens specifically and these modified antigens are not in association with the products of the *K* and *D* loci.

Cold Target Inhibition of I-Region-Directed Cytolysis. Fig. 1 depicts a cold target inhibition experiment of CTL specific for Ia antigens. The lysis produced by AQR anti-6R CTL when tested on a 6R ^{51}Cr-labeled target is significantly inhibited by nonradiolabeled 6R (cold) targets in contrast to the slight level of inhibition seen with AQR TNP-coupled cold targets. These same CTL when

tested on a ^{51}Cr-labeled AQR TNP-modified target are inhibited significantly by both 6R and AQR-TNP cold targets. Therefore, clones which recognize TNP-modified syngeneic Ia determinants are a substantial subset of the clones induced by allogeneic *I*-region stimulation, and lyse targets bearing modified syngeneic Ia antigens specifically.

Discussion and Summary

Data presented in this communication support the conclusions that CTL can recognize *I*-region determinants on TNP-modified syngeneic target cells and that a subset of the clones of CTL induced by allogeneic Ia antigens lyses targets bearing TNP-modified syngeneic Ia antigens. The clones of CTL induced by Ia antigen differences which lyse modified-self Ia antigen-bearing targets are distinct from those clones primarily directed at *K*- or *D*-coded determinants. Thus, varying the *K* or *D* alleles of either the stimulator or target cell does not significantly alter the lysis produced by *I*-region-specific CTL when tested on targets bearing allogeneic or modified syngeneic Ia antigens. Similarly, an antiserum directed at gene products of the *H-2D* locus does not reduce the target lysis by *I*-region-specific CTL. Only an antiserum specific for Ia determinants blocks lysis of CTL induced across *I*-region differences. When tested on modified syngeneic targets, an antiserum specific for syngeneic Ia specificities blocks the lysis of these targets by allogeneically induced CTL, while failing to inhibit the lysis of targets bearing the stimulating allogeneic *I*-region determinants. Added to the cold target data, these observations argue that there exists a significant subpopulation in the clones of CTL induced by allogeneic Ia determinants which recognize and lyse modified syngeneic Ia-bearing cells. These CTL, when presented with targets bearing modified *K*, *I*, and *D* determinants, recognize specifically the modified Ia antigen, though the *K*- and *D*-coded products are the major target antigens in most cytolytic systems (16).

Important issues remain to be investigated concerning these *I*-region-directed clones of CTL. If, as we have suggested, they play an immunoregulatory role, the target of these T cells may be Ia-bearing cells involved in antigen presentation, in macrophage-T cell, T-T cell, or B-T cell interactions, or the factors comprised in part of complexed antigen and Ia determinants which seem to control cell-cell interactions in immune responses. The relationship between those Ia determinants which appear to be concerned with the regulation of the responses to thymus-dependent antigens and those which serve as targets for *I*-region-directed CTL also remains to be elucidated.

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