Brief Definitive Report

CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR *I* REGION DETERMINANTS DO NOT REQUIRE INTERACTIONS WITH *H-2K* OR *D* GENE PRODUCTS*

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Gene products coded for by the major hisocompatibility complex (MHC) can serve as target antigens for cytotoxic T lymphocytes (CTL) (1). A variety of test systems are available which have yielded information consistently reinforcing the importance of this complex of genes in the generation and effector phases of the cytotoxic immune response. Originally, it was shown that allogeneically-induced CTL had specificity primarily for the products of the *K* and *D* loci of the mouse *H-2* complex (2). More recently this has also been found to be the case for xenogeneic immunizations (3, 4). Additional examples of T cell-mediated lysis have been reported involving viral-infected or chemically-modified syngeneic stimulating and target cells in which homology at *H-2K* or *H-2D* was required between the responding and target cells for appreciable lysis to occur (5–7). Moreover, CTL specific for minor histocompatibility antigens are able to lyse only target cells bearing these membrane antigens and sharing a common *H-2K* or *H-2D* gene product with the effector (8, 9).

Two hypotheses have been proposed to explain the requirement for H-2 identity between effector and targets in these systems. CTL may recognize new antigenic determinants created by the interaction of the modifier with syngeneic *K* and *D* gene products. Alternately, a dual recognition system may exist, requiring an antigen-specific receptor as well as a second receptor with specificity for homologous H-2K or H-2D determinants (5). Neither model can be excluded at this time.

The *I* region also contains genes coding for histocompatibility loci since animals differing at the *I-A* or *I-C* regions of the *H-2* complex reject skin grafts (10–12), though less rapidly than mice differing at the *H-2K* or *H-2D* regions. Also CTL can be generated to *I* region determinants but less efficiently than CTL specific for *H-2K* or *H-2D* gene products (12–14). The question can therefore be raised, whether the *I* region minor histocompatibility loci function independently from the *H-2K* or *H-2D* loci or whether *I* region-specific cytolysis requires the participation of *H-2K* or *H-2D* gene products of the target cell.

This communication illustrates the generation of CTL showing specificity for *I* region determinants in primary mixed lymphocyte cultures. Further, we demonstrate by genetic analysis and by the use of specific alloantisera that CTL directed to Ia determinants (*a*) do not see these antigens as modifications of *H-*

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TABLE I
Generation of I Region-Specific Primary Cytolytic Responses by A.TH Spleen Cells In Vitro

Experiment	Stimulator	Target*	Relevant‡ target specificities	Specific release of ^{51}Cr
				%
1	A.TL	A.TL	*I*	55.3
	A.TL	B10.S	None	0.3
2	A.TL	A.TL	*I*	60.5
	B10.BR	B10.BR	*K,I,D*	92.0

* Effector to target ratio was 100:1. Spontaneous release for LPS blasts was 30–38% for A.TL, 37% for B10.BR, and 30% for B10.S.

‡ *H-2*-related specificities which differ for responder and stimulator, but which are shared by stimulator and target, excluding those products of the *S* region.

2K or *H-2D* gene products but as independent gene products coded for by the *I* region, and (*b*) they do not require interaction with target cells bearing the same *H-2K* or *H-2D* gene product as the effector CTL.

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.

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 (15). The ^{51}Cr-release assay used in this study and the method of antisera inhibition have been previously elucidated in detail (16). Lipopolysaccharide-induced (LPS) 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 mediated cytotoxicity (Unpublished results).

Percent-specific release was calculated as:

$$\frac{\text{E-C}}{\text{FT-C}} \times 100\% \quad (16)$$

Results

Generation of I Region-Specific CTL. Several investigators have reported significant cytolysis generated by responder/stimulator pairs differing at the *I* region of the *H-2* complex (12–14). Results shown in Table I confirm this finding. A.TH responder cells stimulated by irradiated A.TL spleen cells lyse LPS induced blast A.TL targets. No significant cytotoxicity could be demonstrated against control B10.S LPS induced blast targets. Significant *I* region killing was always observed and the lytic activity ranged between 12 and 60% in different experiments.

As shown in Table I and Fig. 1, CTL-specific responses to *I* region gene products are weaker than those primarily directed to the *K* and *D* gene products of the *H-2* complex. A.TH responders stimulated by B10.BR spleen cells, a pair

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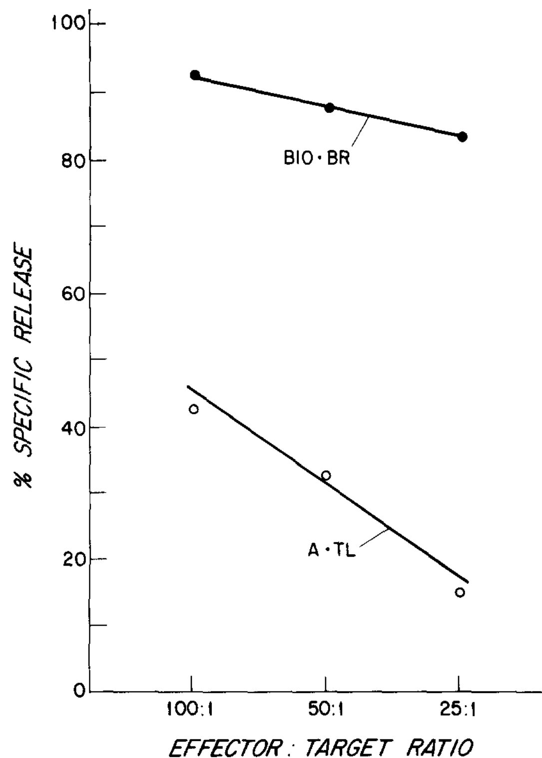

FIG. 1. Lysis of targets bearing relevant *K, I,* and *D* or *I* region determinants by allogeneically induced CTL. A.TH anti-B10.BR effectors were assayed on B10.BR (closed circles) or A.TL (open circles) targets. Spontaneous release for LPS-blast targets was 37% for B10.BR and 30% for A.TL.

which differ for the entire *H-2* complex, kill B10.BR targets more effectively than do A.TH anti-A.TL induced killers, which differ only at the *I* region, when compared at the same effect/target (E/T) ratios. When A.TH responders are stimulated by B10.BR spleen cells and lysis on B10.BR and A.TL targets is compared (Fig. 1), it can be seen that most of the lytic activity generated by this combination appears to be directed to *K* and *D* gene products rather than to *I* region products. From Fig. 1, it can be seen that CTL which react primarily with *I* region determinants (i.e., lyse A.TL targets) comprise fewer than 10% of the allogeneic effector cells.

I Region-Specific CTL Lyse Targets Which Differ at H-2K and/or H-2D From the Stimulator. CTL might recognize Ia antigens as independent cell surface antigens or in relationship with K and D coded molecules. Table II shows two experiments in which A.TH responders were stimulated by spleen cells which differed at the *I* region. The A.TH and anti-A.TL effector cells were then tested on B10.A targets which shared the *I* and *D* regions or B10.BR targets which only share the *I* region with the stimulator cells. A.TH anti-A.TL CTL lyse B10.BR and B10.A targets well and the magnitude of this lysis is comparable to that seen by A.TH anti-A.TL CTL on A.TL targets. Furthermore, as indicated previously, CTL generated in A.TH anti-B10.BR cultures lyse A.TL targets. Thus, *I* region-specific CTL can be efficiently assayed on targets which differ

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TABLE II
I Region-Specific Primary Cytolytic Responses by A.TH Spleen Cells on H-2K and D Incompatible Targets

Experiment	Stimulator	Target*	Shared‡ specificities	Specific release of ^{51}Cr
				%
1	A.TL	A.TL	*K,I,D*	60.5
	A.TL	B10.A	*I,D*	57.5
	A.TL	B10.BR	*I*	54.5
	B10.BR	A.TL	*I*	42.5
2	A.TL	B10.A	*I,D*	17.0
	A.TL	B10.BR	*I*	26.2
	B10.BR	A.TL	*I*	22.1

* Effector to target ratio was 100:1. Spontaneous release of LPS blasts was 30-33% for A.TL, 22-36% for B10.A, and 33-37% for B10.BR.

‡ *H-2*-related specificities shared by stimulator and target, excluding those products of the *S* region.

genetically from the stimulating strain at the *K* and/or *D* loci, i.e., the *I* region antigens appear to be seen as independent cell surface determinants.

Specific Inhibition of I Region Cytolysis by Anti-Ia Antiserum. We next attempted to show that CTL generated to *I* region differences could be specifically blocked by anti-Ia antiserum directed to specificities present on the target. Cytolysis of targets by CTL generated in A.TH anti-A.TL cultures was significantly inhibited by addition of anti-Ia^k antiserum (Table III).

To confirm the observation that Ia antigens act as independent target antigens for CTL, we attempted to inhibit *I* directed killing with an anti-H-$2D^d$ antiserum when both responder and stimulator and responder and target shared the *H-2D*^{d} allele. Thus, A.TH anti-A.TL cultures were assayed on B10.A targets. If *I* region determinants acted as modifiers of *H-2D* gene products then anti-Ia^k antiserum and anti-H-$2D^d$ antiserum should inhibit CTL because stimulator and target bear the same I and D alleles. Likewise, if H-2D homology between responder and target was required to demonstrate *I* region specific CTL, both antisera should significantly inhibit cytolysis. As can be seen in Table III, experiment 2, the anti-Ia^k antiserum inhibited cytolysis to a far greater extent than the anti-H-$2D^d$. The same anti-H-$2D^d$ antiserum very effectively inhibited the lysis produced by CTL generated in allogeneic combinations by using B10 responders and BALB/c stimulators when assayed on these B10.A targets.

Discussion and Summary

CTL specific for determinants coded for by the *I* region of the *H-2* complex can be generated in vitro by sensitization with cells carrying allogeneic *I* region determinants. The detection of *I* region specific CTL is dependent upon testing target cells which express sufficient levels of these determinants, thus we employed LPS blasts as target cells. When analyzing allogeneically induced CTL, the clones reactive with *K* and *D* region products greatly exceed those generated to Ia antigens. It has been demonstrated by genetic analysis and by antibody inhibition that *I* region determinants are recognized independently of

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TABLE III
*Inhibition of I Region Primary Cytolytic Responses by Anti-Ia Antisera**

Experiment	Response	Stimulator	Target‡	Shared specificities§	Antiserum‖		
					Control	αIa^k	αH-2D^d
1	A.TH	A.TL	A.TL	*K,I,D*	60.5	34.5	61.0
	A.TH	A.TL	B10.A	*I,D*	57.5	37.5	65.2
2	A.TH	A.TL	B10.A	*I,D*	17.0	3.8	16.5
	B10	BALB/c	B10.A	*D*	37.2	24.0	13.7

* Values represent percent-specific release of ^{51}Cr.
‡ Effector to target ratio of 100:1. Spontaneous release of LPS blasts was 30% for A.TL and 22–36% for B10.A.
§ *H-2*-related specificities shared by stimulator and target excluding the products of the *S* region.
‖ Control consisted of SMEM-10; (16) anti-Ia^k antiserum is A.TH anti-A.TL with a lytic titer of 1/1280; anti-*H-2D*^{d} is (B10 × LP.RIII)F1 anti-18 R with a lytic titer of >1/640. Both antisera were used at a final dilution of 1:12.

H-2K and *H-2D* gene products. CTL directed to the *I* region determinants lyse all targets bearing the appropriate Ia antigens even if the target cells differ at *H-2K* and/or *D* from the stimulator. An antiserum specific for *I* region antigens significantly inhibits target lysis by CTL, while addition of specific antiserum directed to the *H-2D*^{d} products of the target has little or no affect on the ability of *I*-specific CTL to lyse appropriate Ia bearing targets.

Ia antigens co-cap, redistribute, and immunoprecipitate independently of K and D molecules (17). It now appears that functionally as well, Ia antigens act separately from K and D antigens in their ability to generate specific CTL. Since by all biochemical and functional criteria these Ia antigens are not associated with K and D coded molecules, they appear to represent the products of a third transplantation locus in the mouse MHC as has been suggested by the graft rejection studies of Klein et al. (10, 12).

In light of the findings of Bevan (8) and Gordon, et al. (9), that CTL stimulated by antigens coded for by minor histocompatibility loci or the *H-Y* locus require presentation of these antigens on targets syngeneic at *H-2K* or *D* to the responders, *I* region determinants clearly do not act as other minor histocompatibility loci in this respect.

The *H-2* complex thus codes for at least three molecules which can act as independent CTL stimulating antigens. Viruses, tumor-specific transplantation antigens, chemicals, and minor H antigens appear to behave as modifiers of *H-2K* and *D* determinants, making them recognizable to syngeneic CTL. Studies are now in progress to test whether syngeneic *I* region determinants can also be chemically altered and stimulate CTL in systems which have shown such modifications and activities for *K* and *D* molecules.

Whether there exists a special regulatory role for the relatively few *I* region reacting clones remains unclear. Within the I region, there are genes which regulate many immune responses. The existence of a small number of clones of lymphocytes with receptors which are committed to recognizing *I* region products independently of *H-2K* and *D* products, is suggestive of a possible regulatory role for these cells.

Note Added in Proof. Klein et al. 1977. (*J. Exp. Med.* 145: 450.) have reported similar findings based on genetic evidence since submission of this manuscript.

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References

1. Cerottini, J. C., and R. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. *Adv. Immunol.* **18:**67.
2. Bach, F. H., M. L. Bach, and P. M. Sondel. 1976. Differential function of major histocompatibility complex antigens in T-lymphocyte activation. *Nature (Lond.).* **259:**273.
3. Peck, A. B., B. J. Alter, and K. F. Lindahl. 1976. Specificities in T cell-mediated lympholysis: Identical genetic control of the proliferative and effector phases of allogeneic and xenogeneic reactions. *Transplant. Rev.* **29:**189.
4. Burakoff, S. J., M. E. Dorf, and B. Benacerraf. 1977. Specificity of rat xenogeneic cell-mediated cytolysis for the products of the K and D loci of the mouse H-2 complex. *J. Immunol.* In press.
5. Doherty, P. C., R. V. Blanden, and R. N. Zinkernagel. 1976. Specificity of virus-immune effector T cells for H-2K and H-2D compatible interactions: implications for H antigen diversity. *Transplant. Rev.* **29:**89.
6. Shearer, G. N., T. G. Rehn, and A. Schmitt-Verhulst. 1976. Role of the murine major histocompatibility complex in the specificity of in vitro T cell-mediated lympholysis against chemically-modified autologous lymphocytes. *Transplant. Rev.* **29:**222.
7. Burakoff, S. J., R. N. Germain, and B. Benacerraf. 1976. Cross-reactive lysis of trinitrophenyl (TNP)-derivatized H-2 incompatible target cells by cytolytic T lymphocytes generated against syngeneic TNP spleen cells. *J. Exp. Med.* **144:**1609.
8. Bevan, M. J. 1975. Interaction antigens detected by cytotoxic T cells with the major histocompatibility complex as modifier. *Nature (Lond.).* **256:**419.
9. Gordon, R. D., E. Simpson, and L. E. Samelson. 1975. In vitro cell-mediated immune response to the male-specific (H-Y) antigen in mice. *J. Exp. Med.* **142:**1108.
10. Klein, J., M. Hauptfield, and V. Hauptfield. 1974. Evidence for a third Ir-associated histocompatibility region in the H-2 complex of the mouse. *Immunogenetics.* **1:**45.
11. McKenzie, I. F. C., and M. Henning. 1976. A histocompatibility region (H-21C) in the IC region of the H-2 complex of the mouse. *Immunogenetics.* **3:**253.
12. Klein, J., R. Geib, C. Chiang, and V. Hauptfield. 1976. Histocompatibility antigens controlled by the I region of the murine *H-2* complex. I. Mapping of *H-2A* and *H-2C* loci. *J. Exp. Med.* **143:**1439.
13. Wagner, H., D. Gotze, L. Ptschelinzen, and M. Rollinghoff. 1975. Induction of cytotoxic T lymphocytes against *I*-region-coded determinants: in vitro evidence for a third histocompatibility locus in the mouse. *J. Exp. Med.* **142:**1477.
14. Nabholz, M., H. Young, A. Rijnbeek, R. Boccardo, C. S. David, T. Meo, V. Miggiano, and D. C. Schreffler. 1975. I-region associated determinants: expression on mitogen-stimulated lymphocytes and detection by cytotoxic T cells. *Eur. J. Immunol.* **5:**594.
15. Lemonnier, F., S. J. Burakoff, R. N. Germain, and B. Benacerraf. 1977. Cytolytic T lymphocytes specific for allogeneic stimulator cells cross-react with chemically-modified syngeneic cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:**1229.
16. Burakoff, S. J., R. N. Germain, M. E. Dorf, and B. Benacerraf. 1976. Inhibition of cell-mediated cytolysis of trinitrophenyl-derivatized target cells by alloantisera directed to the products of the K and D loci of the H-2 complex. *Proc. Natl. Acad. Sci. U. S. A.* **73:**625.
17. Klein, J. 1976. An attempt at an interpretation of the mouse H-2 complex. 1976. *Comtemp. Top. Immunbiol.* **5:**297.

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