Brief Definitive Report

VIRUS AND TRINITROPHENOL HAPTEN-SPECIFIC T-CELL-MEDIATED CYTOTOXICITY AGAINST H-2 INCOMPATIBLE TARGET CELLS*

By K. PFIZENMAIER, A. STARZINSKI-POWITZ, H. RODT, M. RÖLLINGHOFF, and H. WAGNER

(From the Institute of Medical Microbiology, 65-Mainz, Hochhaus am Augustusplatz, Germany)

Virus-specific cytotoxic T lymphocytes (CTL) generated in virus-infected mice lyse infected target cells provided they are compatible on either the *H-2K* or *H-2D* gene regions (1–3). A similar *H-2* compatibility requirement has been reported for CTL sensitized against trinitrophenol(TNP)-modified syngeneic spleen cells (4, 5), against minor histocompatibility antigens (6), or the male Y antigen (7). The interpretation of these findings resulted in two mutually exclusive concepts: the "dual recognition" model (1–9) and the "altered self" model (1–9).

To discriminate between both models, we tested the possibility of generating virus-specific or TNP-specific CTL of one *H-2* haplotype against targets of a different *H-2* haplotype. To bypass the problem of alloantigen reactivity, lymphocytes from radiation chimeras (10–11) were used in these studies.

T lymphocytes from irradiated F_1 hybrid mice injected several months previously with T-cell-depleted parental strain bone marrow cells fail to generate CTL reactive towards host type transplantation antigens (11). Since in the mouse the cell-mediated lymphocytes determinants are coded primarily by the *K(D)* region of the *H-2* complex (12), T lymphocytes from F_1 radiation chimeras entirely repopulated with cells of one parental origin are thought to be tolerant against *K(D)*-region products of the other parental *H-2* haplotype.

The results demonstrate that both virus-specific and TNP-specific cytotoxic lymphocytes can be generated against "modified" allogeneic cells. Such CTL do not require *H-2* compatibility between effector cells and target cells for specific lysis to occur.

Materials and Methods

Mice. CBA/J, CBA/J-T6, C57BL/6, and (CBA/J × C57BL/6)F_1 mice from The Jackson Laboratory, Bar Harbor, Maine, and BALB/c mice from Bomholtgard, Ry, Denmark, 8–12 wk of age, were used.

Preparation of Chimeras. 20 semiallogeneic radiation chimeras were prepared as described (10). In short, 2×10^7 viable CBA/J-T6 bone marrow cells were injected intravenously into X-irradiated (900 R) 8-wk-old (CBA × C57BL/6)F_1 mice. Before injection the bone marrow cells were treated with a T-cell specific heterologous antibrain serum plus complement (12). The mice (24 animals) received 100 mg/ml neomycin and 10 mg/liter polymyxin B in their drinking water over a period of 2 wk after irradiation. 20 mice were still alive after 100 days and did not show signs of

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wasting, lack of weight, or other signs indicating a secondary disease. At day 120 postirradiation, a cytogenetic analysis of splenic lymphocytes of three individual mice demonstrated complete chimarism, i.e., all splenic lymphocytes carried the CBA-T6 marker. 5–6 mo postirradiation the radiation chimeras were used for experimental purposes.

Test for Identity of Lymphocytes from the Chimeras. The lymphocytes from each individual chimera used for experimental purposes carried the *H-2*^{k} haplotype according to the following criteria: (*a*) Thymocytes stained 98–100% in the indirect immunofluorescence test using hyperimmune C57BL/6 anti-CBA antiserum and fluorescence isothiocynates-labeled rabbit antimouse Ig serum (Behringwerke, Marburg, Germany). However, they failed to stain with a CBA anti-C57BL/6 antiserum (titer 1:128) followed by fluorescein-labeled rabbit antimouse Ig serum. (*b*) Splenic lymphocytes incubated with C57BL/6 anti-CBA alloantiserum (1:4 dilution) at 37°C, followed by guinea pig complement, were killed to 98–100%, whereas CBA anti-C57BL/6 alloantiserum in the presence of complement did not change the viability of the lymphocytes.

Functional Characteristics of Splenic Lymphocytes of the Chimeras Used. The expression of a permanent, stable tolerance towards parental C57BL/6 alloantigens, of lymphocytes from the chimeras was suggested by the fact that in a mixed lymphocyte culture (MLC) they failed to generate CTL against C57BL/6 alloantigen (unpublished results, see also Table II).

Lymphochoriomeningitis Virus, Immunization and Preparation of Infected Targets. The methods used have been described (3, 13). The only modification introduced was that macrophages were simultaneously infected with LCM virus (WE-3 strain) for 24 h at a multiplicity of infection of about 20 mouse mean lethal dose (LD_{50}) per cell and labeled with [^{51}Cr]chromate (Amersham/Buchler, Frankfurt, W. Germany).

Induction of TNP-Specific Cytotoxic Lymphocytes. The method described by Shearer et al. (4) has been used. In short, X-irradiated (3,000 R) splenic lymphocytes (10^7) were incubated for 10 min at 37°C in phosphate-buffered saline containing 10 mM trinitrobenzol sulfonic acid (TNBS) (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.); 1×10^6 TNP modified cells (stimulator cells) were cocultured over 5 days together with 4×10^6 splenic lymphocytes derived from (CBA × C57BL/6)F_1 chimeras entirely repopulated with CBA/J-T6 lymphocytes. The preparation of lymphoid cells, the culture media, the culture system, and the culture conditions used have been described in detail elsewhere (13, 14).

Preparation of TNP Targets. Replicate cultures of splenic lymphocytes (4×10^6) were cultured in the presence of 5 μg lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Mich.) over a period of 48 h (14). The cells were harvested, labeled with [^{51}Cr]chromate (14) and, if necessary, incubated for 10 min at 37°C in PBS containing 10 mM TNBS. Cells were washed and used as target cells.

Cytotoxicity Assay. Various numbers of viable "attacker cells" (either splenic lymphocytes from infected mice or in vitro sensitized effector cells) were incubated with a constant number (2×10^4) ^{51}Cr-labeled target cells (13, 14). Percent specific ^{51}Cr release was calculated as described (13, 14).

Results

Spleen cells from LCM virus infected (CBA/J × C57BL/6)F_1 radiation chimeras entirely repopulated with lymphoid cells of CBA/J-T6 origin were assayed for virus-specific cytotoxicity against macrophage target cells (Table I). Lysis was recorded both on CBA/J(*H-2*^{k}) and C57BL/6(*H-2*^{b}) mouse-derived LCM virus-infected macrophages. No lysis was observed with noninfected C57BL/6(*H-2*^{b}), CBA/J(*H-2*^{k}), and infected BALB/c(*H-2*^{d}) mouse-derived macrophages. Thus virus-specific lytic interactions could be demonstrated between *H-2* incompatible effector cells and LCM-infected target cells.

Since in the above system the sensitization phase took place in vivo, we next tested the possibility to induce in vitro TNP-hapten-specific cytotoxic lymphocytes against *H-2* incompatible target cells. Spleen cells from (CBA/J × C57BL/6)F_1 radiation chimeras entirely repopulated with CBA/J-T6 lymphocytes were first sensitized in vitro against normal or TNP-conjugated, irradiated CBA/J(*H-*

TABLE I
LCM Virus-Specific Cytotoxic Activity against LCM-Infected Allogeneic Targets

Exp. No.	Percent specific lysis of virus-infected macrophage targets*					
	CBA/J		C57BL/6		BALB/c	
	50:1	5:1	50:1	5:1	50:1	5:1‡
1	74	35	21	5	−2	−3
3	63	30	27	9	0	1
4	37	11	19	4	1	0

Spleen cells of 8-mo-old (CBA × C57BL/6)F_1 radiation chimeras (entirely repopulated with CBA/J-T6 parental lymphocytes, see Material and Methods) immunized i.p. with 10^4 LD_{50} of LCM virus 7 days previously, were tested in a ^{51}Cr release assay for cytotoxicity against LCM infected of normal macrophages. Exp. 1: Background lysis less than 27%, assay time 18 h; Exp. 2: Background lysis less than 24%, assay time 14 h; Exp. 4: Background lysis less than 12%, assay time 6 h.

* Noninfected targets were not lysed.

‡ Ratio of effector cells to target cells used.

2^k), C57BL/6(*H-2*^{b}), and BALB/c(*H-2*^{d}) spleen cells. In a second step the cultured cells were assayed for cytotoxicity against LPS-induced normal or TNP-conjugated blast cells (Table II). TNP-specific cytotoxicity was recorded both against *H-2*^{k} and *H-2*^{b} targets; yet no cytotoxic activity towards normal *H-2*^{b} and *H-2*^{k} targets was demonstrable. Thus similar to the LCM system, TNP-specific lytic interactions could be demonstrated between *H-2*-incompatible cytotoxic effector cells and TNP-modified target cells.

Discussion

The experiments described suggest that virus or TNP-hapten specific cytotoxic lymphocytes can be induced against *H-2*-incompatible modified target cells. Under conditions in which alloantigen reactivity was bypassed by using lymphocytes tolerant to antigens of a given *H-2* haplotype, specific cytotoxicity against allogeneic virus-infected or TNP-conjugated targets was induced. From this it would follow that a state of tolerance against a given *H-2* haplotype appears not to influence T-cell reactivity towards a virus-dependent (or TNP-dependent) "modification" of cell surface structures. Inasmuch as both in the LCM system and in the TNP system the cytotoxic effector cells are shown to be specific for modified gene products coded for by the *H-2K*(*D*) gene regions (1–4), tolerance against "normal" allogeneic *H-2*(*D*) gene products appears to affect different T-cell clones from those reactive against virus-infected or TNP-conjugated allogeneic targets.

So far the *H-2* compatibility requirement reported for virus-specific (1–3) or TNP-specific cytoxicity (4, 5) has been explained by two mutually exclusive concepts. The dual recognition model (1–9) implies two types of receptors. Besides clonally expressed specific receptors, genes mapping at either *H-2K* or *H-2D* region code for "physiological recognition structures," either of which must interact with a complementary "self marker". Therefore *H-2* homology between CTL and targets is thought to be a prerequisite for the lytic effector phase to occur. On the other hand the altered self model (1–9) proposes that

TABLE II

TNP-Specific Cytotoxic Activity Against TNP-Modified H-2 Incompatible Targets

Induction of effector cells		Percent specific lysis of targets (^{51}Cr release assay)									
Responder cells	Stimulator cells (irradiated)	BALB/c*		C57BL/6		C57BL/6-TNP		CBA/J		CBA/J-TNP	
		40:1	4:1	40:1	4:1	40:1	4:1	40:1	4:1	40:1	4:1
(CBA/J × C57BL/6)F_1 radiation chimera entirely repopulated with CBA/J-T6 lymphocytes	BALB/c	62	31	5	−2	ND		ND		ND	
	CBA/J-TNP	1	1	ND		4	1	0	0	48	17
	C57BL/6	ND‡		−1	0	0	0	ND		ND	
	C57BL/6-TNP	1	0	0	0	22	8	−1	0	1	−1

Spleen cells of a 8-mo-old (CBA/J × C57BL/6)F_1 radiation chimera (entirely repopulated with CBA/J-T6 parental lymphocytes, see Material and Methods) were cocultivated with four different types of stimulator cells. After 5 days, the cultured cells were harvested and tested for cytotoxicity against a variety of ^{51}Cr-labeled target cells (LPS-induced blast lymphocytes). Background lysis of the different target cells was less than 19%. Assay time was 3 h. Similar results were obtained with lymphocytes from four individual (CBA/J × C57BL/6)F_1 radiation chimeras tested.

* Ratio effector target cells used.

‡ ND, not done.

virus infection (or hapten conjugation) of cells will result in a specific "modification" of syngeneic *H-2K(D)* gene products which in turn trigger specific clones of antigen-reactive T lymphocytes.

The above demonstration of LCM-specific and TNP-specific cytotoxicity against allogeneic targets argues strongly against the dual recognition model, but is compatible with the altered self concept. In addition, the results suggest that "modifications" of *H-2K(D)* gene products on syngeneic or allogeneic cells are recognized by distinct subsets of antigen-reactive T cells. For example, T lymphocytes sensitized against modified syngeneic cells are able to lyse only *H-2*-compatible modified targets (*H-2* compatibility requirement). On the other hand, T lymphocytes tolerant against the *K(D)* gene products of an allogeneic *H-2* haplotype can be sensitized against modified allogeneic cells. Yet the resultant cytotoxic effector cells exclusively lyse only those modified allogeneic targets, against which they have been sensitized (Table I and II).

In the LCM system or TNP system it was noted that the magnitude of virus-specific or TNP-specific cytotoxic responses towards modified allogeneic cells was significantly lower than that against modified syngeneic cells (Tables I and II). One possible explanation for these findings would be that the T-cell clone reactive against a virus or hapten-induced modification of syngeneic *H-2K(D)* gene products is per se expanded compared to that reactive against a modified allogeneic *H-2K(D)* gene product. This reasoning agrees with the recently proposed view that in terms of immune surveillance a modification of "self" *K(D)* gene products may create antigens against which strong T-cell-mediated cytotoxic immune responses can be mounted (9, 15, 16).

Summary

Immune spleen cells from LCM virus-infected (CBA × C57BL/6)F_1 radiation chimeras entirely repopulated with CBA-T6 lymphocytes were cytotoxic for

allogeneic, LCM virus infected C57BL/6 mouse-derived target cells. Normal C57BL/6 targets were not lysed. CBA-T6 lymphocytes derived from (CBA × C57BL/6) radiation chimeras sensitized in vitro against TNP-conjugated C57BL/6 spleen cells lysed TNP-conjugated C57BL/6 targets. However normal C57BL/6 mouse-derived targets were not destroyed. The magnitude of virus-specific (or TNP-specific) cytotoxic responses against *H-2* incompatible targets was lower compared to that against *H-2* compatible targets. These data are considered to support and to extend the altered self concept, but are not consistent with the dual recognition concept.

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