

Brief Definitive Report

INDUCTION OF VIRUS-SPECIFIC MODIFICATIONS RECOGNIZED BY CYTOTOXIC T CELLS IS NOT ALTERED BY PRIOR SUBSTITUTION OF TARGET CELLS WITH TRINITROPHENOL*

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Cytotoxic thymus-derived lymphocytes (CTL) generated by exposure to cells modified with trinitrophenyl (TNP) groups, or by the process of virus infection, interact maximally with target cells which share both self (*H-2K* or *H-2D*) and nonself (TNP or virus) determinants with the stimulator cell population (1-4). One explanation for this phenomenon is that the T-cell receptor(s) are specific for an "altered-self" component, perhaps a complex of TNP (or virus) and H-2 molecules (1, 5, 6).

Analysis of the virus-immune CTL response of *H-2K* mutant mice indicates that there is no cross-reactivity between the wild-type and two *H-2K* mutant haplotypes (1, 7, 8). Thus, it may be that the *H-2K* component which is recognized by virus-immune CTL may be quite limited in extent, perhaps being modified by mutations which affect only a few amino acid substitutions (1). In contrast, the wild-type and *H-2K* mutant strains exhibit extensive cross-reactivity in the TNP-immune CTL response (9). One interpretation of these contrasting results is that TNP and viral components may form altered-self components by interaction with separate reactive sites on H-2 molecules.

Thorough biochemical studies (10) on TNP-modified cells have shown that the treatment schedules (6, 11) utilized to prepare both stimulator and target cells result in stable substitution of all cell surface H-2 molecules. We thus asked whether prior substitution of the cell membrane with TNP would in any way inhibit the capacity of virus to induce specific cell surface modifications which are recognized by virus-immune CTL. Are reactive sites on H-2 molecules shared between the two systems?

Materials and Methods

Viruses. The influenza type A viruses PR8 [A/PR/8/34 (HON1)], AA [A/Ann Arbor/23/57 (H2N2)], and HK [A/Hong Kong/8/68-X-31 (H3N2)] were supplied and grown as described (12). The WR strain of vaccinia virus was used throughout (13).

Immunizations. Influenza-immune T cells were obtained from the spleens of mice primed with AA, challenged 1 mo later with HK or PR8, and killed after a further 4 days (12). For vaccinia

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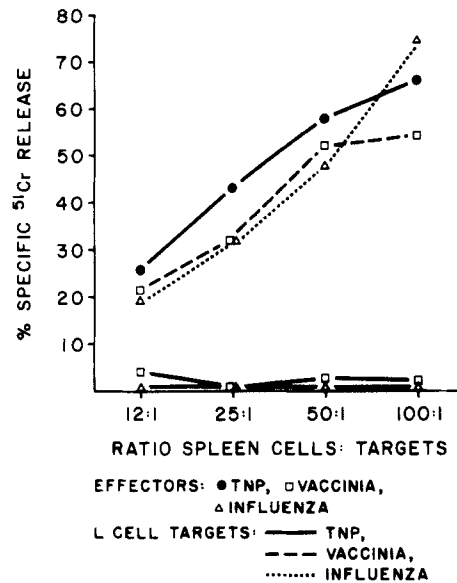


FIG. 1. CTL generated by immunization of CBA/J ($H-2^k$) mice with vaccinia virus or influenza virus lyse virus-infected, but not TNP-modified, H-2-matched target cells. The TNP assays were incubated for 6 h, the virus assays for 12 h. These assays are, at least for the influenza system, approximately equivalent, since maximal antigenic expression on influenza-infected L cells does not occur until at least 5-6 h after infection (see Fig. 3). Spontaneous ^{51}Cr release for all target cells was less than 25%.

immunization, 10^6 mean tissue culture infective doses of vaccinia virus was inoculated intravenously into mice and their spleens were harvested 6 days later (13). TNP-immune CTL were generated by culturing normal spleen cells for 5 days in vitro with irradiated TNP-modified syngeneic spleen cells as described by Burakoff et al. (11).

Cytotoxicity Assay. Cytotoxicity against ^{51}Cr -labeled influenza- and vaccinia-infected L929 fibroblasts (C3H L cells, $H-2^k$) and MC57G sarcoma cells (C57BL/6, $H-2^b$) was measured as outlined in detail elsewhere (12). For TNP modification, $1-2 \times 10^7$ cells were reacted with 150 μl of 2.5 mM trinitrobenzene sulfonic acid in phosphate-buffered saline, pH 7.2, for 10 min at 37°C , washed, and labeled with $\text{Na}_2^{51}\text{CrO}_4$. Results are expressed as percent specific ^{51}Cr release (12) and are the means of quadruplicate determinations.

Results and Discussion

The results in Fig. 1 and Table I demonstrate that TNP-modified L cells are lysed by TNP-immune but not influenza- or vaccinia-immune H-2 compatible lymphocyte populations. Target cells which were first modified with TNP and then infected with influenza virus were killed by both TNP- and influenza-immune CTL (Table I), but not by H-2 incompatible virus-immune effector cells. Furthermore, competitive inhibition experiments clearly demonstrated that anti-TNP and anti-influenza CTL were specific for the antigens used for sensitization (Fig. 2).

Therefore, saturation of the plasma membrane with TNP does not detectably inhibit the capacity of influenza virus to infect cells or to induce the expression of antigen(s) which are recognized by influenza-immune CTL. These results do not simply reflect binding of the influenza virus inoculum to which the cells

TABLE I
T cells Recognize Two Distinct Neoantigens on One Target Cell

Mouse strain	Immune lymphocytes*	% Specific ^{51}Cr release†				
		L cells (<i>H-2^b</i>)				MC57G (<i>H-2^b</i>)
		Normal	TNP	TNP + HK	HK	HK
CBA/J (<i>H-2^b</i>)	TNP	16	84	100	19	1
	Influenza 1	0	1	41	52	0
	Influenza 2	0	2	76	66	0
C57BL/6 (<i>H-2^b</i>)	Influenza 1	1	2	6	3	25
	Influenza 2	3	3	7	5	39

* TNP-immune effector cells were obtained from 5-day in vitro cultures of CBA spleen cells and irradiated TNP-modified CBA spleen cells. Influenza-immune T cells were obtained from mice primed 1 mo previously with AA, challenged with (group 1) A/PR8/1934 (HON1) or (group 2) HK and sampled 4 days later. Effector cell:target cell ratios were 20:1 for TNP-immune effectors and 40:1 for virus-immune effectors.

† Normal or TNP-modified target cells were infected with HK \times 31 for 1 h at 37°C and then labeled with $\text{Na}_2^{51}\text{CrO}_4$. All assays were then incubated for 12 h at 37°C. The standard error of the mean of quadruplicate determinations was always less than 5% of the mean.

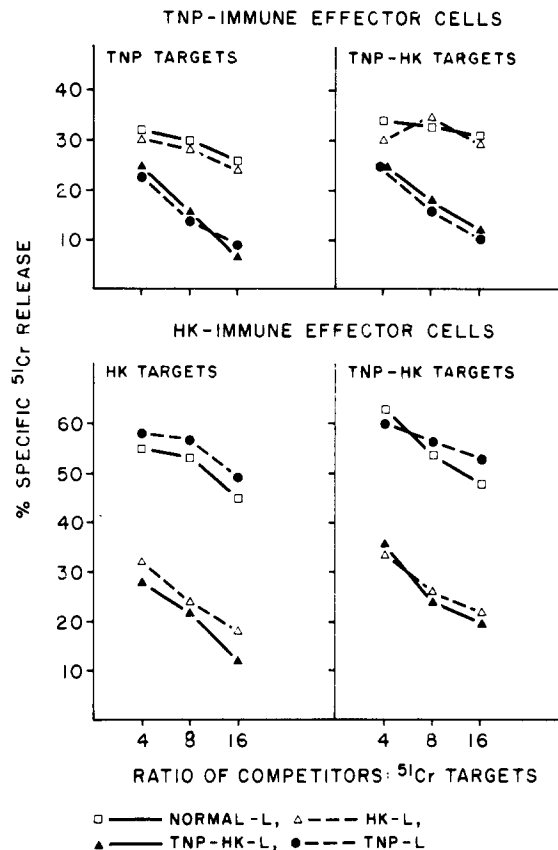


FIG. 2. Competitive inhibition with unlabeled target cells indicates that different T-cell populations mediate lysis directed against viral and TNP antigenic determinants expressed on the same target cells. Effector cell: ^{51}Cr -labeled target cell ratios were 10:1 for TNP-immune effectors and 50:1 for HK-immune effectors. All assays were incubated for 12 h.

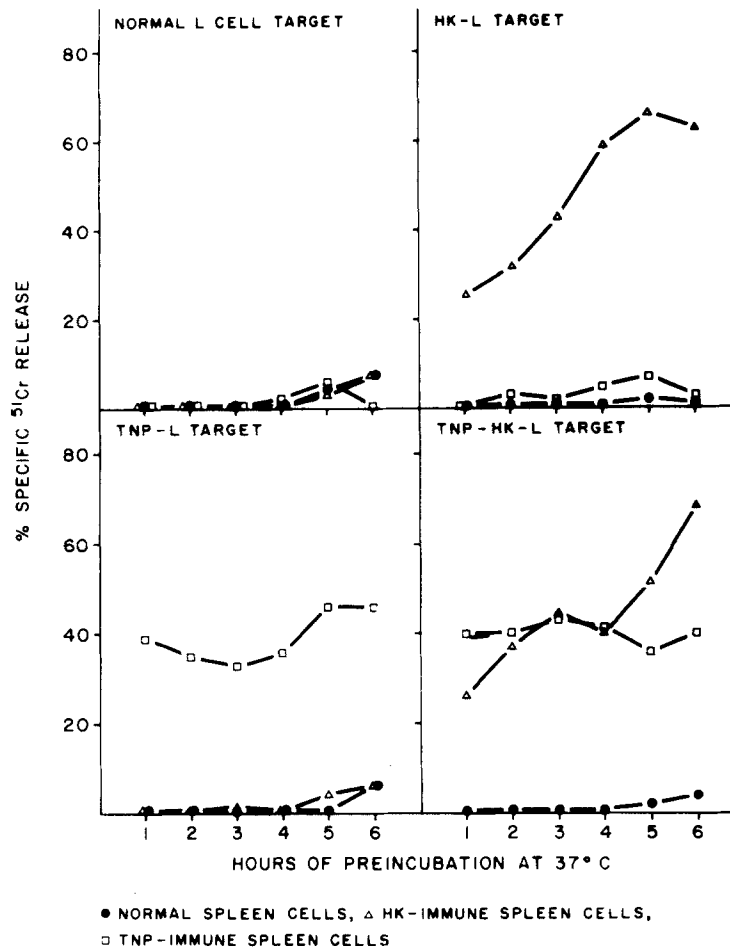


FIG. 3. Normal or TNP-modified L cells were infected with influenza virus for 1 h at 37°C, washed once, dispensed into assay plates, and held at 37°C. Lymphocyte populations were added at times corresponding to 1-6 h after exposure to the virus, and incubated for a further 6 h at 37°C. Effector cell:target cell ratios were 35:1 for TNP-immune effectors and 50:1 for normal and HK-immune effectors.

were exposed, since maximal antigenic expression at the plasma membrane is only observed 5-6 h after exposure to virus (Fig. 3). Also, since influenza virus is a potent inhibitor of host cell protein synthesis (14), it is unlikely that any new H-2 antigen is synthesized.

Starzinski-Powitz et al. (15) reported that cells first infected with lymphocytic choriomeningitis virus (LCMV) and then modified with TNP could be killed by both TNP- and LCMV-immune CTL. However, LCMV might not be expected to "modify" all available H-2 molecules on these cells, since viral antigen is not expressed at a particularly high concentration on the cell membrane (P. C. Doherty, unpublished observations). Also, Starzinski-Powitz et al. (15) observed that anti-vaccinia and anti-LCMV CTL lysed TNP-modified target cells almost as effectively as did anti-TNP CTL. Therefore, it could not be determined if TNP

modification of virus-infected cells altered the induction of virus-specific modifications recognized by virus-immune CTL.

In the present study, we have been unable to demonstrate any lysis of TNP-modified target cells by CTL from mice immunized with either vaccinia or influenza virus. Target cells which were modified with TNP and then infected with influenza virus expressed distinct cell surface antigens recognized by TNP- and influenza-immune CTL. These results indicate that if altered-self antigens exist as a complex between nonself and H-2 (16), there is probably more than one reactive site on the H-2 molecule. If TNP- and virus-immune CTL do not recognize TNP molecules or viral components but are reactive to specific conformational changes in *H-2K* or *D* molecules induced by these agents, *H-2K* or *D* molecules must then be able to simultaneously express at least two distinct alterations. An alternative (17-19) is that individual T cells express two distinct receptors with specificity for self (H-2) and nonself (virus or TNP).

Summary

Cytotoxic thymus-derived lymphocytes generated after interaction with trinitrophenyl (TNP)-substituted or virus-infected cells only lyse H-2 compatible target cells modified with the component used to immunize (TNP or virus). Prior saturation of TNP-reactive sites inhibits neither the infectivity of influenza A viruses, nor the capacity of infected cells to develop antigenic changes recognized by influenza-immune T cells. The two antigens are distinct entities on the cell membrane and do not obviously compete to form interactions with H-2 molecules.

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References

1. Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-immune effector T cells for H-2K or H-2D compatible interactions: implications for H-antigen diversity. *Transplant. Rev.* 29:89.
2. Pfizenmaier, K., A. Starzinski-Powitz, H. Rodt, M. Rölinghoff, and H. Wagner. 1976. Virus and trinitrophenol hapten-specific T-cell-mediated cytotoxicity against H-2 incompatible target cells. *J. Exp. Med.* 143:999.
3. Zinkernagel, R. M. 1976. Virus-specific T-cell-mediated cytotoxicity across the H-2 barrier to virus-altered alloantigen. *Nature (Lond.)* 261:139.
4. Von Boehmer, H., and W. Hass. 1976. Cytotoxic T lymphocytes recognize allogeneic tolerated TNP-conjugated cells. *Nature (Lond.)* 261:141.
5. Zinkernagel, R. M., and P. C. Doherty. 1974. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature (Lond.)* 248:701.
6. Shearer, G. M., T. G. Rehn, C. A. Garbarino. 1975. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. *J. Exp. Med.* 141:1348.
7. Zinkernagel, R. M. 1976. H-2 compatibility requirement for virus-specific T-cell-mediated cytolysis. The H-2K structure involved is coded by a single cistron defined by H-2K^b mutant mice. *J. Exp. Med.* 143:437.
8. Blanden, R. V., M. B. C. Dunlop, P. C. Doherty, H. I. Kohn, and I. F. C. McKenzie. 1976. Effects of four H-2K mutations on virus-induced antigens recognized by cytotoxic T cells. *Immunogenetics.* 3:541.

9. Forman, J., and J. Klein. 1977. Immunogenetic analysis of H-2 mutations. VI. Cross-reactivity in cell-mediated lympholysis between TNP-modified cells from H-2 mutant strains. *Immunogenetics*. 4:183.
10. Forman, J., E. S. Vitetta, D. A. Hart, and J. Klein. 1977. Relationship between trinitrophenyl and H-2 antigens on trinitrophenyl-modified spleen cells. I. H-2 antigens on cells treated with trinitrobenzene sulfonic acid are derivatized. *J. Immunol.* 118:797.
11. Burakoff, S. J., R. N. Germain, M. E. Dorf, and B. Benacerraf. 1976. Inhibition of cell-mediated cytotoxicity 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.
12. Effros, R. B., P. C. Doherty, W. Gerhard, and J. Bennink. 1977. Generation of both cross-reactive and virus-specific T cell populations following immunization with serologically distinct influenza A viruses. *J. Exp. Med.* 145:557.
13. Koszinowski, U., and H. Ertl. 1975. Lysis mediated by T cells and restricted by H-2 antigen of target cells infected with vaccinia virus. *Nature (Lond.)*. 255:552.
14. Lazarowitz, S. G., R. W. Compans, and P. W. Choppin. 1971. Influenza virus structural and nonstructural proteins in infected cells and their plasma membranes. *Virology*. 46:830.
15. Starzinski-Powitz, A., K. Pfizenmaier, U. Koszinowski, M. Rölinghoff, and H. Wagner. 1976. Shared determinants between virus-infected and trinitrophenyl-conjugated H-2-identical target cells detected in cell-mediated lympholysis. *Eur. J. Immunol.* 6:630.
16. Schrader, J. W., B. A. Cunningham, and G. M. Edelman. 1975. Functional interactions of viral and histocompatibility antigens at tumor cell surfaces. *Proc. Natl. Acad. Sci. U. S. A.* 72:5066.
17. Doherty, P. C., D. Götze, G. Trinchieri, and R. M. Zinkernagel. 1976. Models for recognition of virally modified cells by immune thymus-derived lymphocytes. *Immunogenetics*. 3:517.
18. Zinkernagel, R. M., and P. C. Doherty. 1977. The concept that surveillance of self is mediated via the same set of genes that determines recognition of allogeneic cells. *Cold Spring Harbor Symp. Quant. Biol.* 41:505.
19. Janeway, C. A., H. Wigzell, and H. Binz. 1976. Two different V_H gene products make up the T-cell receptors. *Scand. J. Immunol.* 5:993.