

ANTIGEN RECOGNITION BY H-2-RESTRICTED  
CYTOLYTIC T LYMPHOCYTES IS NOT MEDIATED  
BY TWO INDEPENDENT RECEPTORS

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A major dilemma in contemporary immunology is the question of how T lymphocytes are able to demonstrate dual specificity for both nominal antigen and the products of the major histocompatibility complex (MHC) such that both antigens must be presented on the same cell for antigen recognition to occur (1). Two major models have been proposed to explain this observation. The dual receptor model states that T cells possess separate receptors, one for nominal antigen and one for MHC products (2–4). In the original version of this model, these two receptors were postulated to be independent of one another (1). In contrast, the altered-self hypothesis states that T cells express only one receptor, which recognizes a new antigenic product produced by the interaction of these two structures (5–8). Inconclusive evidence has been accumulated to support each of these models but proves neither.

In the accompanying paper (9), we have demonstrated that it is possible to solubilize cloned cytolytic T lymphocytes (CTL) and transfer specific cytolytic activity to other cells not expressing this property. This transfer was enacted through the Sendai virus-mediated fusion of liposomes (derived from donor CTL) to the recipient cells. In the present report, we have used this system to investigate the specificity of fusion products obtained between two non-cross-reactive H-2-restricted CTL clones. The results demonstrate that both specificities of the parental clones (i.e., nominal antigen plus H-2) were demonstrated in the fusion product; however, no evidence for new antigenic specificities (as would be predicted by independent reassociation of receptors in the dual receptor model) was obtained.

**Materials and Methods**

The materials and methods used in this study were as previously described in the accompanying paper (9). A few of the methods require further details.

*Target Cells.* For the cytolytic assays, the target cells consisted of the following tumor cell lines: P815 (H-2<sup>d</sup>, carcinogen-induced), LSTRA (H-2<sup>d</sup>, Moloney leukemia virus [MoLV]-induced), and MBL-2 (H-2<sup>b</sup>, MoLV-induced). In addition lipopolysaccharide-induced spleen cell blasts of (H-2<sup>b</sup>) C57BL/6 (B6) male, B6 female, (H-2<sup>d</sup>) BALB/c (B/c) male, and B/c female origin were used as target cells.

*CTL Clones.* CTL clones used in these experiments were derived by micromanipulation as described in detail elsewhere (10, 11). Cytolytic clone 7 was derived from a peritoneal exudate lymphocyte of a B6 mouse immunized against P815 cells. Clone 14 is a B6

cytolytic clone directed against MoLV antigenic determinants in association with H-2D<sup>b</sup>. Clone H-Y is a B6 clone with cytolytic specificity for the male (H-Y) antigen in association with H-2D<sup>b</sup>. Clone MoLV is a B/c CTL clone specific for MoLV antigenic determinants in association with H-2K<sup>d</sup>.

*Preparation of Liposomes.* Liposomes were prepared by a modification of published procedures (12–14) as described in the accompanying paper (9). Briefly, donor cells were solubilized in Nonidet P-40 detergent-containing buffer. After removal of nuclei and mitochondria by low speed centrifugation, the supernatant (containing matrix and membrane proteins) was added to detergent-solubilized Sendai virus envelope glycoproteins (hemagglutinin/neuraminidase [HN] and fusion [F]) and exogenous lipids (dipalmitoyl-phosphatidylcholine [DPPC] and cholesterol) to construct liposomes. Liposomes were derived by 48-h dialysis at 4°C against a 1,000-fold excess of phosphate-buffered saline (PBS). Large liposomes (average size, 0.5–10 μm) were collected by high speed centrifugation (50,000 g, 1 h) and washed with PBS in this manner.

*Cellular Reconstructions.* Liposomes and recipient cells were incubated together for 1 h at 4°C to allow HN-mediated liposome/cell binding. After washing, the cells were incubated for 30 min at 37°C to allow F-mediated liposome/cell fusion. At the end of this time, the cells were washed and used immediately in the assays. All fusions were performed at a ratio of two cell equivalents of liposomes to recipient cells; i.e., in a typical experiment,  $1 \times 10^6$  recipient cells were fused with liposomes derived from  $2 \times 10^6$  donor cells. Cell equivalents are defined as described (9). Designation of the experimental protocol is in the form "F(donor)/recipient," which indicates that liposomes derived from a specific donor were fused to the indicated recipient.

## Results and Discussion

In the accompanying paper (9), we demonstrated that it was possible to solubilize and transfer specific cytolytic activities from cloned CTL to noncytolytic T cell clones, T cell tumor lines, and a B cell line, with the aid of liposomes. To apply this technology to the question of receptor specificity in H-2-restricted CTL, it was first necessary to demonstrate that CTL with "double" specificity could be constructed by fusing liposomes derived from CTL clones with other CTL clones that possessed another distinct antigenic specificity. In one such experiment, liposomes were constructed from a B6 (H-2<sup>b</sup>) anti-MoLV CTL clone (clone 14) and fused with a B6 (H-2<sup>b</sup>) anti-P815 (H-2<sup>d</sup>) CTL clone (clone 7). As shown in Fig. 1, the fusion product that we obtained [F(14)/7] exhibited cytolytic activity against both P815 and MBL-2 (H-2<sup>b</sup>, MoLV) target cells, while the original CTL clones were active against only one or the other of these target cells. When this experiment was performed in converse, similar results were observed (Fig. 1). That is, if liposomes were derived from clone 7 and fused with clone 14, the fusion product [F(7)/14] was now able to lyse both the P815 and MBL-2 target cells. These experiments are significant in that they extend our original observations to include CTL clones (both alloreactive and H-2 restricted) as suitable recipients for liposomal fusion.

Having established that fusions such as those described above were possible, we next attempted to investigate whether H-2-restricted CTL recognize antigen in the context of MHC products displayed on a target cell by one or two receptors. To do this, we derived non-cross-reacting clones specific for either H-Y antigen in the context of H-2<sup>b</sup> (clone H-Y) or MoLV in the context of H-2<sup>d</sup> (clone MoLV), constructed liposomes from each clone, and fused these liposomes with the other CTL clone. We then examined the fusion products for their ability to lyse each of the target cells susceptible to the parental CTL clones

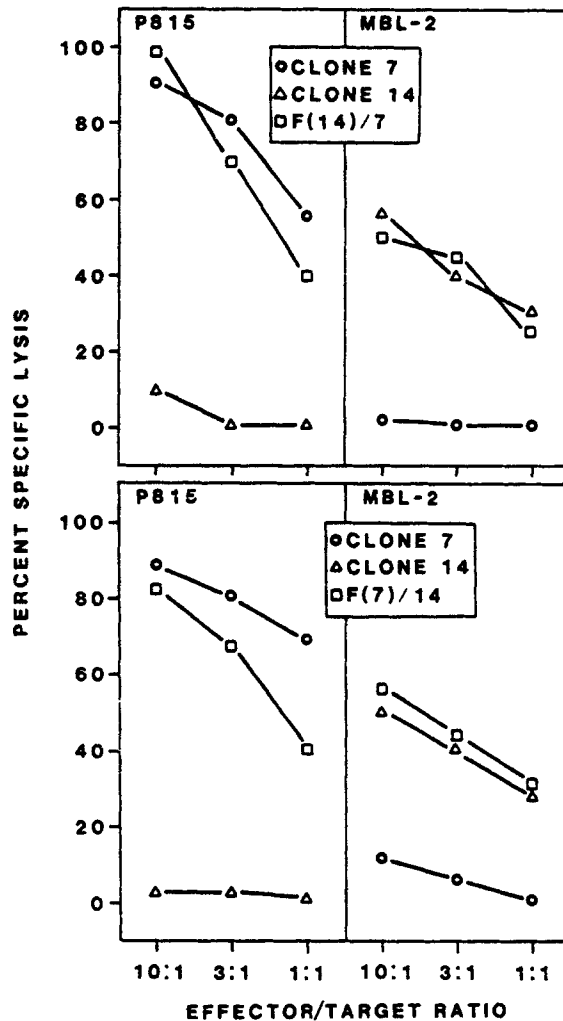


FIGURE 1. Creation of CTL clones with dual specificity by liposome-mediated fusion. (*Top*) Liposomes derived from clone 14 (B6 anti-MoLV) were fused with clone 7 (B6 anti-H-2<sup>d</sup>). The resulting fusion product [designated F(14)/7], as well as the two parental CTL clones, were then tested for cytolytic activity against either P815(H-2<sup>d</sup>) or MBL-2(H-2<sup>b</sup>-MoLV) target cells at the indicated effector/target ratios in a 2-h <sup>51</sup>Cr-release assay. (*Bottom*) The reciprocal experiment was performed; i.e., liposomes derived from clone 7 were fused with clone 14 and tested for cytolytic activity as described above.

(H-2<sup>b</sup>-H-Y and H-2<sup>d</sup>-MoLV target cells) and asked whether these fusion products would now express a new "recombinant" antigenic specificity, namely react with H-2<sup>d</sup>-H-Y and/or H-2<sup>b</sup>-MoLV target cells. The results of two of these experiments are shown in Table I. Regardless of the fusion product constructed [either F(MoLV)/H-Y or F(H-Y)/MoLV] these CTL only displayed lytic activity against the target cells recognized by the original parental clones. That is, the reconstructed CTL could lyse both LSTRA (H-2<sup>d</sup>-MoLV) and B6 male (H-2<sup>b</sup>-H-Y) target cells but were not active against any of the other target cells. If new recombinant antigenic specificities had been produced, according to the dual

TABLE I  
*Specificity of Fusion Products Obtained Between Two H-2-restricted CTL Clones*

Experiment	Effector population	Target cells*					
		MBL-2 (H-2 <sup>b</sup> - MoLV)	LSTRA (H-2 <sup>d</sup> - MoLV)	B6 ♂ (H-2 <sup>b</sup> - H-Y)	B6♀ (H-2 <sup>b</sup> )	B/c ♂ (H-2 <sup>d</sup> - H-Y)	B/c ♀ (H-2 <sup>d</sup> )
1	Clone H-Y	2	2	100	0	0	0
	Clone MSV	2	80	0	7	0	3
	F(MoLV)/H-Y <sup>‡</sup>	5	86	94	9	4	3
	F(H-Y)/MoLV <sup>‡</sup>	6	82	62	1	8	5
2	Clone H-Y	13	4	82	6	8	5
	Clone MSV	8	89	11	10	2	6
	F(MoLV)/H-Y <sup>‡</sup>	4	100	78	7	7	7
	F(H-Y)/MoLV <sup>‡</sup>	5	89	62	8	8	5

\* Data are expressed as the percent specific lysis obtained in a 2-h cytotoxicity assay at an effector/target ratio of 10:1 for each of the individual targets.

<sup>‡</sup> Liposomes were derived from a clone specific for H-2<sup>d</sup>-MoLV and fused with a clone specific for H-2<sup>b</sup>-H-Y at a ratio of 2:1 cell equivalents.

<sup>‡</sup> Liposomes were derived from a clone specific for H-2<sup>b</sup>-H-Y and fused with a clone specific for H-2<sup>d</sup>-MoLV at a ratio of 2:1 cell equivalents.

receptor hypothesis, it would have been expected that either MBL-2(H-2<sup>b</sup>-MoLV) or B/c male (H-2<sup>d</sup>-H-Y) target cells would have been lysed. These experiments argue very strongly against the possibility that H-2-restricted CTL recognition is mediated by two independent antigen receptors. However, it cannot be excluded that such recognition is mediated by two closely linked (and hence not freely reassociable) antigen receptors. It should be noted that our observations agree with those recently published by Kappler et al. (15) who found similar results upon somatic cell fusion of interleukin 2-producing, H-2-restricted, non-cytolytic hybridomas with distinct antigen specificities. They also failed to observe any recombinant antigenic specificities after such fusions. However, this latter system is more difficult to interpret in that the functional hybrids produced are tetraparental and therefore chromosome loss and/or contributions from the T lymphoma fusion partners possibly could influence the results. In contrast, the liposomal transfer system described herein is rapid, does not depend upon the introduction of selectable genetic markers into cloned CTL, and does not (with the exception of artificial lipids and Sendai virus F protein) introduce any non-CTL-derived material into the fused cells.

In conclusion, our results provide the strongest evidence obtained to date that H-2-restricted CTL do not recognize antigen via two independent receptors. Furthermore, the liposomal transfer system described herein provides an experimental framework for the future analysis and purification of putative receptor molecules on T lymphocytes (16-18) that is independent of any a priori assumptions as to their structure.

### Summary

Detergent-solubilized murine cytolytic T lymphocytes (CTL) clones were incorporated into Sendai virus-containing synthetic liposomes. When these liposomes were then fused with other CTL clones possessing a different non-cross-reacting specificity, the fusion products were observed to lyse target cells recognized by both parental CTL clones. This method was then used with two H-2-

restricted CTL clones of different, non-cross-reacting specificities (anti-H-2<sup>b</sup>-H-Y or anti-H-2<sup>d</sup> Moloney leukemia virus). Once again, the fusion products were found to be lytic against both target cells recognized by the parental clones, but in no instance was there any observable lysis of target cells bearing the same nominal antigen in the context of different H-2 molecules. These results provide strong evidence that antigen recognition by H-2-restricted CTL is not mediated by two independent antigen receptors.

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### References

1. Zinkernagel, R. M., and P. C. Doherty. 1975. H-2-compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus: different cytotoxic T-cell specificities are associated with structures coded from H-2K or H-2D. *J. Exp. Med.* 141:1427.
2. von Boehmer, H., W. Haas, and N. K. Jerne. 1978. Major histocompatibility complex-linked immune responsiveness is acquired by lymphocytes of low responder mice differentiating in thymus of high responder mice. *Proc. Natl. Acad. Sci. USA.* 75:2439.
3. Blanden, R. V., and G. L. Ada. 1978. A dual recognition model for cytotoxic T cells based on thymic selection of precursors with low affinity for self-H-2 antigens. *Scand. J. Immunol.* 7:181.
4. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882.
5. Zinkernagel, R. M., and P. C. Doherty. 1977. Major transplantation antigens, virus, and specificity of surveillance T cells. The "altered self" hypothesis. *Contemp. Top. Immunobiol.* 7:179.
6. Rosenthal, A. S. 1978. Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* 40:136.
7. Paul, W. E., and B. Benacerraf. 1977. Functional specificity of thymus-dependent lymphocytes. *Science (Wash. DC).* 195:1293.
8. Hünig, T. R., and M. J. Bevan. 1982. Antigen recognition by cloned cytotoxic T lymphocytes follows rules predicted by the altered-self hypothesis. *J. Exp. Med.* 155:111.
9. Harris, D. T., H. R. MacDonald, and J.-C. Cerottini. 1984. Direct transfer of antigen-specific cytolytic activity to noncytolytic cells upon fusion with liposomes derived from cytolytic T cell clones. *J. Exp. Med.* 159:261.
10. Glasebrook, A. L., M. Sarmiento, M. R. Loken, D. P. Dialynas, J. Quintans, L. Eisenberg, C. T. Lutz, D. Wilde, and F. W. Fitch. 1981. Murine T lymphocyte clones with distinct immunological functions. *Immunol. Rev.* 54:225.
11. MacDonald, H. R., R. P. Sekaly, O. Kanagawa, N. Thiernesse, C. Taswell, J. C. Cerottini, A. Weiss, A. L. Glasebrook, H. D. Engers, K. T. Brunner, and C. Bron. 1982. Cytolytic T lymphocyte clones. *Immunobiology.* 161:84.
12. Hale, A. H., O. N. Witte, D. Baltimore, and H. N. Eisen. 1978. Vesicular stomatitis virus glycoprotein is necessary for H-2-restricted lysis of infected cells by cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 75:970.
13. Hale, A. H., M. J. Ruebush, D. T. Harris, and P. P. McGee. 1981. Elicitation of anti-H-2 cytotoxic T lymphocytes with antigen-modified H-2-negative stimulator cells. *J. Immunol.* 126:1485.

14. Prujansky-Jakobovits, A., D. J. Volsky, A. Loyter, and N. Sharon. 1980. Alteration of lymphocyte surface properties by insertion of foreign functional components of plasma membranes. *Proc. Natl. Acad. Sci. USA.* 77:7247.
15. Kappler, J. W., B. Skidmore, J. White, and P. Murrack. 1981. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. *J. Exp. Med.* 153:1198.
16. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Murrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149.
17. Reinherz, E. L., S. C. Meuer, and S. F. Schlossman. 1983. Delineation of antigen receptors on human T lymphocytes. *Immunol. Today (Amst.)*. 4:5.
18. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen-specific human T cell function. Relationship to the T3 molecular complex. *J. Exp. Med.* 157:705.