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COMMUNICATION

The Induction of Cytolytic T Lymphocytes with Syngeneic Trinitrophenyl-Coupled Membranes[1]

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Recently we have demonstrated the induction of allogeneic murine cytolytic T lymphocytes (CTL)[3] using purified plasma membranes rather than intact cells as stimulatory agents (1). In this report we extend the use of such subcellular preparations to study the requirements for hapten-specific syngeneic CTL induction. Membranes prepared from trinitrophenyl (TNP) coupled syngeneic tumor cells retain the ability to stimulate both a primary and secondary CTL response. The CTL that are generated are restricted in their lysis to target cells bearing the same H-2 antigens as those present on the TNP-coupled stimulating membranes.

MATERIALS AND METHODS

All materials and methods used in the *in vitro* induction and assay of TNP specific CTL are as previously described (2). Briefly, 7×10^6 spleen cells from nonimmune or immune mice were co-cultured with x-irradiated, TNP-coupled spleen cells or TNP-coupled membranes. After 5 days of culture cells were harvested and cytolytic activity was assessed in a 4-hr assay against 10^4 ^{51}Cr-labeled TNP-coupled tumor targets or LPS blast cell targets. Immune spleen cells were obtained by priming mice subcutaneously with 2×10^7 TNP-coupled autologous spleen cells 2 weeks before *in vitro* culture. Membranes used in stimulation of CTL were prepared from TNP coupled DBA/2 mastocytoma P815 (H-2^d) tumor cells or from TNP-coupled C57BL/6 (B6) leukemia EL-4 (H-2^b) tumor cells. Purified plasma membranes were used for CTL induction in the experiment described in Table I. The results presented in Tables II and III were obtained by using partially purified plasma membranes referred to as "high speed pellet" in Reference 1. Spontaneous ^{51}Cr release ranged from 30 to 39% for LPS-induced blast cell targets and from 11 to 19% for tumor cell targets.

RESULTS

Plasma membranes prepared from TNP-modified tumor cells were tested for their ability to stimulate both primary and secondary CTL responses by using H-2 syngeneic responder cells. As demonstrated in Tables I and II, such membranes were active in the stimulation of primary and secondary hapten specific CTL. CTL generated by coupled membranes are similar in specificity to those generated by coupled cells in that they preferentially lyse syngeneic target cells (2–4). These membranes stimulated variable amounts of cross-reactive lysis on TNP coupled allogeneic target cells. B6 spleen cells stimulated with TNP-EL-4 membranes did not lyse targets that did not bear TNP. Lytic activity was not induced when B6 spleen cells were co-cultured with uncoupled EL-4 membranes.

It has been recently demonstrated (5, 6) that cells incubated with TNP-coupled proteins are capable of stimulating a hapten-specific cytolytic response that is restricted to target cells that are H-2 identical with the responder cell population. Therefore, it was important to determine if stimulation by the membranes was dependent on the H-2 antigen present on the membrane, or whether the membrane proteins were simply contributing the hapten that was then recognized in conjunction with the H-2 antigens of the responder cell population. B6D2F$_1$ (H-2$^{b/d}$) immune spleen cells were stimulated with either TNP-EL-4 membranes or TNP-P815 membranes and the specificity of the resultant CTL was studied. It would be expected that if the H-2 present on the membranes did not influence the specificity of the CTL, then in either case the CTL would lyse both B6-TNP (H-2^b) and B10.D2-TNP (H-2^d) targets to a similar extent. As is shown in Table III the CTL preferentially lyse target cells that bear the same H-2 antigens as the TNP-membranes used in CTL stimulation. Similar specificity was obtained with CTL resulting from stimulation of nonimmune B6D2F$_1$ spleen cells (Table II). These results indicate that both the TNP and the H-2 antigens present on the membranes determine the specificity of the CTL population.

DISCUSSION

The results described above extend the use of subcellular material to the study of CTL recognition in a chemically modified syngeneic system. The results demonstrate the capacity of membranes prepared from TNP-modified tumor cells to induce primary and secondary CTL having the same specificity as CTL that are induced by TNP-coupled cells. The ability to stimulate B6D2F$_1$ CTL that are restricted in their recognition to the H-2 antigens present on the stimulating membrane

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[3] Abbreviations used in this paper: CTL, cytolytic T lymphocytes; H-2, histocompatibility complex-2; B6D2F$_1$, (C57BL/6 × DBA/2)F$_1$; B6, C57BL/6.

TABLE I

Specificity of secondary (BALB/c × DBA/2)F_1 (H-2^d) CTL stimulated by TNP-coupled membranes

Stimulator[a]	% Specific ^{51}Cr Release			
	P815-TNP (H-2^d)		EL-4-TNP (H-2^b)	
	25/1[b]	12.5/1	25/1	12.5/1
Experiment 1				
—	19	9		
12-μg membranes	32	19		
24-μg membranes	44	24		
72-μg membranes	59	38		
Experiment 2				
—	31	19	19	12
BALB/c-TNP cells	83	61	40	14
3-μg membranes	31	16	13	8
10-μg membranes	41	19	17	11
30-μg membranes	63	40	22	14

[a] Membranes used for this experiment were purified plasma membranes obtained from TNP-coupled P815 tumor cells (1).

[b] Effector to target ratio.

TABLE II

Induction of primary (C57BL/6 × DBA/2)F_1 (H-2$^{b/d}$) CTL by TNP-coupled membranes

Stimulator[a]	% Specific ^{51}Cr Release[b]	
	B6-TNP (H-2^b)	B10.D2-TNP (H-2^d)
—	7	7
B6-TNP cells	64	34
24-μg membranes	28	12
75-μg membranes	33	6
150-μg membranes	38	6

[a] Membranes used in this experiment were partially purified from TNP-coupled EL-4 tumor cells (H-2^b).

[b] Effector to target ratio is 50:1. Target cells were LPS-stimulated blast cells.

TABLE III

Specificity of TNP-membrane-induced CTL

Responder	Stimulator	% Specific ^{51}Cr Release Targets[a]	
		B6-TNP (H-2^b)	B10.D2-TNP (H-2^d)
Primed B6D2F_1 (H-2$^{b/d}$)	—	6	9
	75 μg EL-4-TNP membranes	29	10
	84 μg P815-TNP membranes	16	32

[a] Effector to target ratio was 50:1. Targets were LPS-stimulated blast cells.

preparations indicates that induction results from recognition of both the H-2 and the hapten on the membranes and not from haptenated protein(s) from the membranes that associate with the responder cells. In this regard, TNP membranes are similar in their inductive capacity to TNP cells. It has been previously reported that H-2 antigens need not be directly haptenated in order to obtain a CTL response. Recent experiments that have utilized TNP-coupled serum proteins to stimulate TNP-specific CTL have argued against the contention by Forman *et al.* (7) that only TNP present on H-2 is antigenically active. Although the experiments described above do not address the question of a requirement for direct haptenization of H-2 to stimulate CTL, it is clear that TNP-membranes are antigenically similar to TNP-cells rather than TNP-proteins.

It is also of interest to consider these results as they address the mechanism of CTL stimulation by subcellular material. The possibility exists that stimulation of CTL by subcellular preparations occurs via presentation of antigen by intact cells present in the cultures (e.g., macrophages) rather than by direct interaction between the membrane vesicle and pre-CTL. It is clear that if indeed material must be presented by viable cells to be antigenic, these cells do not determine the specificity of the resulting CTL.

Ozata and Henney (6) have reported that membranes from TNP-coupled spleen cells failed to induce a secondary syngeneic CTL response whereas the results shown in Tables I and II of this report clearly show that membranes from TNP-coupled tumor cells can induce a specific secondary response. This discrepancy might be accounted for by the difference in cell type used as a membrane source.

The ability to stimulate CTL with TNP-modified membranes opens the possibility that we will be able to isolate, in a soluble form, TNP-modified membrane proteins that retain biologic activity (i.e., the ability to induce CTL) (8). One could then determine whether an effective immunogen is created by TNP-modified non-MHC proteins that interact with H-2, or whether direct chemical modification of H-2 antigens creates the immunogen, or whether both possibilities exist.

SUMMARY

Evidence is presented that trinitrophenyl-coupled tumor membranes are able to induce cytolytic T lymphocytes (CTL) when co-cultured with syngeneic spleen cells. These haptenated membranes stimulate spleen cells from naive and immune mice. The specificity of these CTL is determined by the H-2 antigens of the membranes used for stimulation.

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