Brief Definitive Report

EVIDENCE FOR DIRECT LINKAGE BETWEEN ANTIGEN RECOGNITION AND LYTIC EXPRESSION IN EFFECTOR T CELLS*

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The mechanism of cytotoxicity mediated by thymus-derived (T) lymphocytes can be divided into two phases, one of antigen recognition, the other the cytolytic event. The antigen recognition phase shows immunological specificity and consists of the interaction of target cell antigen with the effector cell's antigen receptor site (1-3). The relationship between this accomodation and the subsequent killing process is unknown.

Conceptually, one could view the role of the T cell's antigen receptor in one of two ways. On the one hand, the receptor could serve simply as a bridge, bringing the target cell into close approximation with the effector cell. It could be that the duration of this contiguity is an essential element in rendering the target cell susceptible to lytic attack. In this model, activation of the killing mechanism would be independent of antigen recognition, either because the differentiated effector cell can cause lysis without requiring activation, or because the "triggering" required occurs at sites of cell-cell interaction which have no linkage with the antigen receptor.

On the other hand, the receptor site might play a much more central role in a T cell's lytic attack, and there might be a direct linkage between antigen binding and stimulation of the lytic mechanism. This model would suggest that the killer cell only expresses its lytic potential after accomodation of its antigen receptor.

In both hypotheses, the antigen receptor site endows specificity to T-cell-mediated lysis, but the models assign markedly different roles to the receptor and view cytolysis in distinctive ways. The experiments reported here were designed to decide between these alternative concepts. The studies were based on a recent observation that lytically active T cells can themselves serve as targets for other effector T cells (4, 5). We asked a rather simple question: if one mixed effector cells of two specificities, selected so that antigen recognition could occur only in one direction (Fig. 1), did killing occur in one or both directions? Our findings provided an unequivocal answer: cytolysis proceeded only in the direction of antigen recognition. The effector cell with its antigen receptor site

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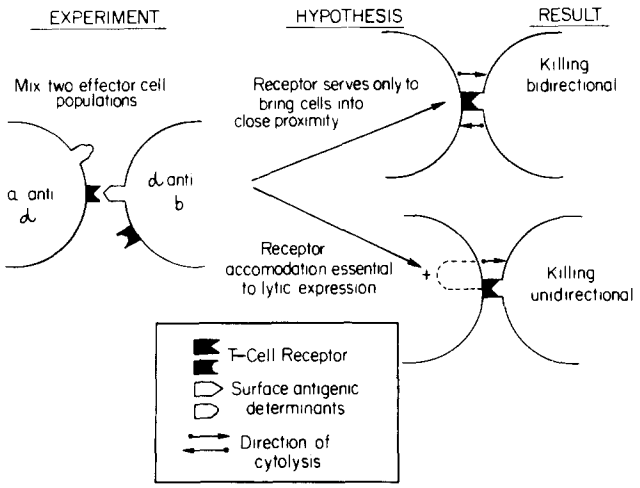

FIG. 1. Schematic representation of possible roles of the antigen receptor in T-cell-mediated lysis.

occupied exerted its lytic activity, the other effector cell did not. This finding implies that accomodation of the effector T cell's antigen receptor is necessary for the expression of lytic activity.

Materials and Methods

Effector Cell Populations. B10.A SgSn and B10.D2 nSn mice (The Jackson Laboratory, Bar Harbor, Maine) were injected intraperitoneally with 3×10^7 spleen cells from either B10.D2 nSn or C57BL/10 mice, respectively. 10 days later, single spleen cell suspensions were prepared as previously described (6). The resulting cell suspension was washed twice in RPMI 1640 (Microbiological Associates, Bethesda, Md.), containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol (RPMI-2ME). Viability (erythrocin B dye exclusion) was invariably higher than 75%.

6×10^7 B10.A or B10.D2 primed spleen cells were then cultured in RPMI-2ME in the inner chamber of a Marbrook glass vessel (Bioresearch Glass, Vineland, N. J.) together with 12×10^6 mitomycin C-treated (50 μg/ml, 30 min, 37°C) B10.D2 or C57BL/10 spleen cells, respectively. The vessels were harvested after culture for 2–5 days. The cells were resuspended to 10^7 viable cells/ml in RPMI-2ME. All cell-mediated cytotoxicity was removed by treatment (7) with an AKR anti-Thy-1 antiserum in the presence of rabbit complement.

Assessment of Cytolytic Activity. The cultured cells were assayed separately in microtiter plates (8) against ^{51}Cr-labeled P815 mastocytoma (DBA/2 derived, *H-2*^{d}) and against ^{51}Cr-labeled EL4 lymphoma (C57BL/6 derived, *H-2*^{b}). Control assays employed normal spleen cells instead of immune populations.

At the end of the assay the released ^{51}Cr was counted (8). Percent specific lysis was calculated by the formula:

$$\frac{^{51}\text{Cr immune lymphocytes} - {}^{51}\text{Cr normal lymphocytes}}{\text{Freeze-thaw lysis} - {}^{51}\text{Cr normal lymphocytes}} \times 100,$$

as described by Brunner et al. (9).

Results

B10.A spleen cells sensitized to B10.D2 cells (a anti-d cells) killed P815-X2 (*H-2*^{d}) target cells with virtually no cross-reactivity being exhibited towards EL4 lymphoma cells (*H-2*^{b}). On the other hand, B10.D2 effector cells raised against

TABLE I

The Effect of Coculturing Effector Lymphocyte Populations on Their Subsequent Lytic Activity

Spleen cells cocultured*		Ratio of B10.A cells to B10.D2 cells in coculture	Residual specific activity after coculture			
B10.A (a)	B10.D2 (d)		% Specific ^{51}Cr release against *H-2*^{d} cells	% Reduction in a anti-d activity	% Specific ^{51}Cr release against *H-2*^{b} cells	% Reduction in d anti-b activity
Immune§ (a anti-d)	Normal		17.6	0‡	0	–
Normal	Immune (d anti-b)	1:1	0.8	–	63.7	0‡
Immune (a anti-d)	Immune (d anti-b)		16.3	7	39.1	39
Immune (a anti-d)	Normal		27.2	0‡	3.8	–
Normal	Immune (d anti-b)	1:0.25	0	–	53.0	0‡
Immune (a anti-d)	Immune (d anti-b)		31.4	0	4.9	98

Spontaneous release of ^{51}Cr from EL4 target cells was 13.1% and from P815 cells 8.2%. In both cases, freeze-thawing target cells released 80% ± 1% of the incorporated ^{51}Cr.

* 2×10^6 B10.A spleen cells were cultured (6 h, 37°C) with either normal or immune B10.D2 spleen cells at ratios of 1:1 or 1:0.25. Lytic activity was then assessed in a 4 h assay using 10^4 ^{51}Cr target cells of either *H-2*^{b} (EL4) or *H-2*^{d} (P815) specificities and 4×10^5 lymphoid cells. These multiplicities were based on the total number of "a" lymphoid cells initially added to the cultures.

‡ By definition.

§ The a anti-d populations were B10.A spleen cells raised against B10.D2 spleen cells. The d anti-b populations were B10.D2 spleen cells raised against C57BL/10 spleen cells (see Materials and Methods for details).

C57BL/10 alloantigens (d anti-b cells) killed EL4 cells but not P815 cells (Table I).

The effect of coculturing these two effector populations was assessed by incubating the cells together for 6 h at 37°C at a multiplicity of 1:1 or 1:0.25 "a" cells to "d" cells. Control cultures contained either normal a or normal d spleen cells instead of the effector populations. At the end of culture the cells were added to ^{51}Cr-labeled EL4 or P815 target cells to give a lymphocyte:target cell multiplicity of 40:1 and lysis was assessed 4 h later.

As can be seen from Table I, the anti-d effector cells maintained comparable levels of activity after incubation with either normal or effector d-cell populations (17.6 vs. 16.3% after incubation at 1:1 ratio, 27.2 vs. 31.4% at a 1:0.25 ratio). The absolute levels of activity reflected the number of competitive d lymphocytes present when ^{51}Cr-labeled d cells (P815) were added to the culture. Predictably, there was less inhibition when a 1:0.25 ratio of a to d cells was used in the initial incubation.

In contrast, the d anti-b effector cells lost considerable activity on culture with a anti-d effector cells, but not when cultured with normal a cells. At a ratio of 1:1, the fall in lytic activity of the d anti-b population at the end of 6 h was from 63.7 to 39.1% (a 39% decline). An almost total loss (53 down to 4.9%) of d anti-b activity was seen when the effector cells were cocultured at an a-cell to d-cell ratio of 1:0.25.

Similar experiments using effector cells of different specificities gave comparable results (Table II). In all cases, the inactivation of effector cells proceeded only in the direction of antigen recognition: there was never reciprocal effector cell death. It is clear, therefore, that when two lytically active cell populations are mixed in circumstances in which antigen recognition can occur only in one

TABLE II

Further Experiments on the Effect of Coculturing Effector Cell Populations

Exp.	Effector cell combination in coculture		Ratio effector I:II	% Reduction in lytic activity after coculture	
	Effector population I	Effector population II		Effector population I	Effector population II
A	B10.A anti-B10.D2	B10.D2 anti-C57BL/10	1:1	4	39
B	B10.A anti-B10.D2	B10.D2 anti-C57BL/10	1:0.25	0	94
C	C57BL/6 anti-P815	A/J anti-Chang	1:1	6	85
D	C57BL/6 anti-P815	DBA/2 anti-L	1:2	15*	98
E	C57BL/6 anti-P815	DBA/2 anti-L	1:0.25	0	91
F	DBA/2 anti-EL4	C57BL/6 anti-L	1:0.25	0	96

An experimental design identical to that employed in Table I was used. After coculture for 6–18 h lytic activity was assayed using appropriate target cells at a lymphocyte:target cell ratio of 100:1. In the experiments shown, the specific lysis of homologous target cells never varied more than twofold (range 31–60%).

* In this experiment, in keeping with known serological specificities, the DBA/2 anti-L-cell population showed some cytotoxicity towards C57BL/6 spleen cells.

direction, then the activity of that population exhibiting the antigen is lost while cells bearing receptors for the antigen retain their lytic function.

Discussion

It is clear from this report, and from the earlier findings of Golstein (4), and of Martz (5), that effector T cells can serve as target cells for other killer lymphocytes. Further, we infer on the basis of this study that when an effector cell serves as a target, it does not reciprocally kill the cell which causes its demise. This conclusion is based on, and is limited by, the following considerations. If reciprocal cell death occurred after effector cell-effector cell interaction, then it is apparent that the fall in lytic activity in both populations would be stoichiometrically related, and the extent of cell death would be a function of effector cell incidence in the interacting populations. Thus, if the number of effector cells in the two populations was the same, the decline in activity would be identical. Similarly, if there were twice as many effectors in the a anti-d population than in the d anti-b, then the death of "a" effector cells would occur half as frequently as the death of "d" killer cells. The only circumstance that would preclude measurement of reciprocal cell death would be one in which the number of a anti-d effectors greatly exceeded that of d anti-b effectors. (In this case the killing of a cells would be very much lower than that of d cells.) In fact, we observed little or no fall in the lytic activity of the a anti-d population when virtually all of the b effector cells were inactivated (Tables I and II). Thus, either there was no reciprocal cell death, or the a effector cells greatly exceeded the d effector cells. Although there is, as yet, no reliable indicator of the absolute number of effector cells present in a lytically active cell population, it is a common practice to consider that populations with comparable levels of cytolysis have a similar incidence of effector cells (10). As the cytolytic activity of the a anti-d and d anti-b cell populations towards homologous tumor target cells was comparable in the studies reported here, we assume that the effector cell incidence in the attacking and target populations did not markedly differ. We thus conclude that there is no reciprocal cell death after effector cell-effector cell interaction.

The studies reported in Tables I and II involving a number of effector cells with different specificities indicate that when antigen recognition occurs in only one direction, then the cell whose antigen receptor site is accomodated functions as the killer cell, and the cell whose antigen is inserted becomes the target cell. Further, in preliminary experiments which will be reported in detail elsewhere, we have demonstrated that inactivation of both killer cell populations does occur in situations where antigen recognition occurs in both directions (e.g., BL/6 anti-DBA/2 mixed with DBA/2 anti-BL/6).

It is interesting to note in Table I an apparent anomaly between the cytolytic activity of the effector cell populations towards ^{51}Cr-labeled tumor cells and their capacity to inactivate allogeneic effector cells. During a 4 h incubation period the a anti-d effector cells lysed 27% of ^{51}Cr-labeled P815 cells at a lymphocyte:target cell ratio of 40:1. There was, however, an almost total inactivation (98%) of the d anti-b effector cell activity during the 6-h incubation at a multiplicity of 1:0.25. It is not clear whether this finding indicates that effector T cells are more readily lysed than are homologous tumor cells, or whether inactivation of the effector cell precedes the destruction of its membrane.

It would thus appear that antigen-recognition site accomodation is essential to the expression of lytic activity by the effector T cell. The killer cell does not, therefore, seem to be an intrinsically lytic cell, or one triggered simply by the proximity of another cell, but one whose potential to exert cytolysis is controlled by its receptor site. This does not rule out nonreceptor associated "triggers," but it does suggest that the antigen receptor plays a much more central role in the lytic event then merely serving as a bridge between effector and target cell. The precise role of the receptor remains, of course, to be determined: the site might itself house the killing mechanism or might be allosterically linked to it. Alternatively, receptor site-associated enzymes might act as "second messengers" controlling lytic expression.

Summary

The relationship between antigen recognition and lytic expression by effector T cells was examined by coculturing two cytolytically active lymphoid cell populations. When antigen recognition between the populations could occur only in one direction, then cytotoxicity was expressed only in that direction and the population whose antigens were recognized lost its lytic activity. In contrast, the cocultured effector cell population fully maintained its lytic potential. This lack of reciprocal inactivation was taken as evidence that T-cell receptor accomodation by surface antigen is linked to the expression of cytolytic activity by effector T lymphocytes.

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