Brief Definitive Report

SELF-SPARING OF LONG-TERM IN VITRO–CLONED OR UNCLONED CYTOTOXIC T LYMPHOCYTES

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When they lyse target cells, cytotoxic T lymphocytes are not themselves lysed, since they are able to further lyse other target cells. The apparently simple question of how the effector cells manage to avoid lysis has been a long-standing puzzle. One explanation is that, after recognition-triggered activation of the effector cells, lysis is still somehow polarized towards the target cell; for instance, the T cell receptor might not only ensure recognition, but also direct the mechanism of lysis. A second explanation would be a nonpolarized mechanism, such as the release by the effector cell of cytolytic structures in its environment, to which the effector cell should then be resistant. If the latter mechanism is the mechanism of T cell–mediated cytotoxicity, then cytotoxic T cells should be resistant to T cell–mediated cytotoxicity.

An early series of experiments, making use of the short-term, uncloned cytolytic T cell populations available at the time, showed that when A anti-B cells were incubated with B anti-C cells, the latter underwent a strong decrease in their ability to lyse C target cells (1, 2). This was interpreted as lysis of B anti-C effector cells by A anti-B effector cells, thus indicating that effector T cells were not intrinsically resistant to lysis; similar results and conclusions were also reached in lectin-dependent T cell–mediated cytotoxicity (3). This interpretation imposed a need for a polarized, unidirectional effector-to-target mechanism of lysis, thus raising serious constraints on, for instance, hypotheses based on the secretion by the effector cells of nonspecifically cytotoxic molecules.

We show here that at least some cloned cytolytic T cells that, in line with the previous findings, are able in the presence of lectin to lyse (and be lysed by) other cytolytic T cells, are unable to lyse themselves. This phenomenon (*a*) can account by itself for the survival of a given cloned effector cell when it lyses, thus lifting in this case any formal requirement for polarity of the mechanism of lysis, (*b*) is also found with long-term in vitro–cultured uncloned cytotoxic T cell populations, and (*c*) is, in turn, itself in need of an explanation.

Materials and Methods

Cells. The following cytotoxic cells were used either as effector cells or as ^{51}Cr-labeled target cells: clones KB5 C20 (B10.BR anti-B10, anti–class I, dependent for growth on

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TABLE I

A Cytolytic T Cell Clone Lyses Other Cells, Is Lysed by Polyclonal Cytolytic Cell Populations, but Does Not Lyse Itself

^{51}Cr-labeled target cells	Con A during test	Percent specific ^{51}Cr release with effector cells at E/T ratios of:									Spontaneous release
		KB5C20			Anti-H-2^b			Anti-H-2^k			
		20	5	1	20	5	1	20	5	1	
KB5C20	−	0	1	0	4	1	0	57	30	10	10
	+	0	0	0	53	35	13	53	29	9	13
RDM4	−	1	0	2	14	4	2	68	45	22	6
	+	52	26	13	64	45	22	69	47	29	6
EL4	−	82	78	65	83	70	49	7	4	0	7
	+	75	69	50	80	78	56	72	72	55	10

Effector cells were either the T cell clone KB5C20 or 5-d MLC cells (H-2^k anti-H-2^b or H-2^b anti-H-2^k). ^{51}Cr-labeled target cells were the same T cell clone, or lymphomas RDM4 (H-2^k) or EL4 (H-2^b). The cytotoxicity test was for 4 h at 37°C in the presence or absence of Con A. Results are presented as percent ^{51}Cr release minus spontaneous release of target cells alone.

allostimulation and IL-2 [4]) and A15.1.17 (A.TH anti-A.TL, anti–class II, dependent for growth on allostimulation and IL-2 [5]) both restimulated on day 0 and on day 7 with irradiated allogeneic cells and IL-2, and on day 4 with IL-2 alone, and used as target or effector cells on day 7 or 8; hybridomas FLH1.25.5.S and SPH1.3 (anti-H-2^k–fluorescein and anti-H-2^k-SP [SP is 3-(*p*-sulfophenyldiazo)-4-hydoxyphenyl acetic acid] hapten, respectively, constitutive for growth and cytotoxicity [6]); the three latter clones also lysed RDM4 in the absence of Con A, and to a lesser extent EL4 cells, see Table II; uncloned C57BL/6, B10, or B10.BR spleen cells maintained in culture either under Con A stimulation (Miles Laboratories, Elkhart, IN) (triple crystallized, 1.5 μg/ml) or under allostimulation (γ-irradiated spleen cells), with addition of IL-2 after the first passage in both cases. In all instances, IL-2 was added in the form of supernatants of EL4 C116 stimulated with PMA (7). Lymphomas EL4 and RDM4 were also used as target cells.

Cytotoxic Tests. We used conventional 4-h ^{51}Cr-release tests in microplate wells at the indicated E/T ratios. Some experimental groups also received Con A (usually 10 μg/ml), or leukoagglutinin (Leuko A; Pharmacia Fine Chemicals, in a range of concentrations from 0.6 to 10 μg/ml) or PMA (0.8×10^{-6} M; Sigma Chemical Co., St. Louis, MO).

Results

In one experiment, the results of which are given in Table I, we used as ^{51}Cr-labeled target cells the cytolytic T cell clone KB5C20, and as control target cells, we used the two lymphomas EL4 and RDM4; we used as effector cells the same cytolytic T cell clone, and as control effector cells, anti-H-2^b or anti-H-2^k 5-d mixed leukocyte culture (MLC) cells. In the absence of Con A, KB5C20 and anti-H-2^b cells significantly lysed EL4 target cells, and anti-H-2^k cells significantly lysed KB5C20 (of B10.BR, i.e., H-2^k, origin) and RDM4 target cells. In the presence of Con A, each of the effector cells, including KB5C20 lysed RDM4 and EL4, and KB5C20 was lysed by the MLC cells. Thus, KB5C20 could lyse and be lysed; most importantly however, it did not lyse itself, even in the presence of Con A.

In another experiment, the results of which are given in Table II, four cytolytic T cell clones or hybridomas were used as effector and target cells, with additional control RDM4 and EL4 target cells. In the presence of Con A, the latter was lysed by each of the effector cells. Also, each of these four clones or hybridomas

TABLE II

Cytolytic T Cell Clones Lyse Other Cells, Are Lysed or Not by Certain Other Cytolytic T Cell Clones, but Do Not Lyse Themselves

^{51}Cr-labeled target cells	Con A during test	Percent specific ^{51}Cr release with effector cells				Spontaneous release
		KB5C20	A15.1.17	FLH-25.5.S	SPH1.3	
KB5C20	−	0	1	0	3	10
	+	1	4	57	47	12
A15.1.17	−	4	4	5	0	21
	+	2	−1	42	30	34
FLH1.25.5.S	−	2	−1	−2	2	29
	+	1	2	0	2	31
SPH1.3	−	3	3	0	−1	29
	+	2	0	3	0	30
RDM4	−	2	50	56	48	11
	+	27	33	76	45	11
EL4	−	65	16	18	19	11
	+	66	48	76	69	10

Effector cells and ^{51}Cr-labeled target cells (but for RDM4 and EL4) were all cytotoxic T cells clones or hybridomas. E/T ratio was 20. The experimental conditions and presentation of results were otherwise as in Table I.

could be lysed by MLC effector cells (not shown). Thus, each of the four cloned effector cells could lyse and be lysed; also, some of them lysed others (i.e., FLH-1.25.5.S and SPH1.3 lysed KB5C20 and A15.1.17) or did not (i.e., KB5C20 lysed neither itself nor the three other clones). Most importantly, except for A15.1.17, which exhibited some lysis upon mere addition of Con A (see in Table II the increase in spontaneous release when Con A was added), none of them lysed itself.

This lack of self-lysis was very reproducible. With KB5C20 in the presence of Con A, it occurred in each of 15 independent experiments. It was not limited to Con A–dependent cytotoxicity, since it was also observed in the presence of a range of concentrations of Leuko A or in the presence of PMA (not shown). However, in some cases, for instance again for A15.1.17 in Table II and for a subline of KB5C20 (especially at higher concentrations of Con A, not shown), the mere addition of Con A increased spontaneous release, which may or may not be due to Con A–dependent cell-mediated cytotoxicity. These particular cases could therefore not be interpreted, as opposed to the absence of self-lysis clearly shown by the other clones. We recently observed (not shown) that not only cytotoxic T cell hybridomas, but also AB2, a cytotoxic T cell clone obtained through the courtesy of Dr. W. R. Clark (Molecular Biology Institute, University of California, Los Angeles, CA), was able to lyse very efficiently the cytotoxic clone KB5C20 in the presence of Con A; again, AB2 did not lyse itself under the same conditions.

We wondered whether the apparent absence of self-lysis observed in Tables I and II might be linked to the loss, during the 4-h cytotoxicity test in the presence of Con A, either of the ability to lyse or of the ability to be lysed. Experiments (not shown) in which KB5C20 cells were tested again after a 4-h test in the presence of Con A, either as killers or as targets (with other cytotoxic cells),

TABLE III

Long-term Polyclonal Cytotoxic T Cells Do Not Lyse Themselves

^{51}Cr-labeled target cells	Con A during test	Percent specific ^{51}Cr release from effector cells at E/T ratios of:				Spontaneous release
		(b anti-k)$_5$		(Con A blast)$_8$		
		8:1	2:1	4:1	1:1	
(b anti-k)$_5$	−	1	0	0	3	30
	+	3	2	3	1	31
(Con A blast)$_8$	−	−3	−2	−2	−4	31
	+	−1	−1	−1	−3	33
KB5C20	−	70	50	51	11	4
	+	65	48	54	20	12
EL4	−	60	23	60	12	7
	+	69	51	74	28	8
RDM4	−	51	59	57	26	18
	+	51	54	52	35	14

(b anti-k)$_5$, B6 spleen cells stimulated for 5 d in a primary MLC with irradiated CBA spleen cells, restimulated weekly with irradiated CBA spleen cells plus IL-2-containing supernatant, and tested 5 d after the fourth restimulation (after 32 days in culture). (Con A blast)$_8$, B6 spleen cells stimulated for 2 d with Con A (1.5 μg/ml) restimulated twice weekly with Con A (1.5 μg/ml) plus IL-2-containing supernatant, and tested 2 d after the seventh restimulation (after 25 days in culture).

showed that they had lost neither of these abilities. These results also showed that absence of self-lysis was demonstrable not only in terms of lack of ^{51}Cr-release, but also in terms of conservation of cytolytic function (which was the experimental outcome in the earlier experiments [1–3] with primary effector cell populations).

The effector cells used in the experiments above were cloned, and therefore had to be grown for a long time in vitro. We wondered how long-term in vitro polyclonal effector cell populations would behave in the same sort of experiments. Table III shows that such lines were very efficient at lysing EL4 and RDM4 target cells and also KB5C20 in the presence or even in the absence of Con A (this lack of specificity raising some questions as to the exact nature of the effector cells in this case), but most important, these lines were resistant to self-lysis.

Discussion

We have shown that at least some cloned cytotoxic T cells spare themselves in the presence of Con A, although under the same experimental conditions they can lyse, and be lysed by, other cytotoxic T cells. These results, which in previous studies (1–3) were obscured by polyclonality and/or the use of primary rather than long-term in vitro–grown cells, raise at least three points.

The results do not require a polarized mechanism of cytotoxicity. There is no such formal requirement, and no logical constraint against a mechanism of lysis based on, for example, soluble factors acting at short range. It should be strongly emphasized that this conclusion is valid only for in vitro–cultured cloned and long-term uncloned cytotoxic T cells such as those used here, or such as those used in a recent study (8) where, in line with the present results, cloned cytolytic T cells were not lysed when exerting direct or backwards killing. We do not

know yet whether the same conclusion would also apply to individual primarily activated effector cells, which by definition cannot be obtained as clones; experimental evidence for self-sparing may not be easy to obtain at the single- (dual-) cell level.

The results were obtained not only with clones, but also with long-term uncloned cytotoxic T cell populations. These should be equivalent to the sum of many clones, which might therefore interkill in the presence of Con A, but did not. One possibility would be that these populations have become truly monoclonal, which we do not think very likely. A more intriguing possibility, suggested in particular by the hierarchy of resistance observed (see the Tables) would be that there exists a process in culture, somehow selecting cells that may be at the same time the most efficient killers and the most lysis-resistant targets within the available cell population. When the available starting cell population is polyclonal, the level of resistance and lytic activity finally reached would have most chances to be higher than when the starting cell population is monoclonal. This hypothesis would account for the observation that cloned cells are lysed by polyclonal cells. It also leads to the testable prediction that clones made from fresh lymphoid cell populations should have different levels of resistance to lysis, while clones made from long-term–cultured cell populations should be more uniformly resistant to most killer cell clones or populations. From another point of view, we do not know whether the same evolution to resistance would occur in vivo.

The results lead to a new question: how can some T cells resist lysis by some effector T cells and not by others, under conditions (lectin-mediated lysis) that seem to preclude any selectivity of cell recognition? Perhaps a first step to answering this question will be to investigate whether there are quantitative or qualitative clonal differences in effector or target molecules at play in different cytotoxic T cell clones.

Summary

At least some long-term in vitro–cultured cytotoxic T cell clones and uncloned cell populations are able, in the presence of Con A, to lyse other cells, to be lysed by other cells, but not to lyse themselves. This as-yet-unexplained result may have implications as to the mechanism of T cell–mediated cytotoxicity.

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