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A Combination of Soluble Helper Factors Bypasses the Requirement for Stimulator Cells and Induces Nonspecific Cytotoxic T Cell Responses

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Abstract

The specificity of cytotoxic T lymphocyte (CTL) responses generated in the presence of lymphokines was studied. Thymic responder cells were activated in the presence of stimulator cells that differed in their metabolic activity. After 5 days of culture, the cytotoxic response was estimated in a 4-h ^{51}Cr-release test. Coculture of thymic responders with irradiated splenic stimulator cells in the presence of interleukin 2 (IL 2) led to preferential cytolysis of target cells that expressed the same histocompatibility antigens as the cells used for sensitization. Addition of T cell cytotoxicity-inducing factor 1 (TCF1), however, to those cultures made the presence of stimulator cells unnecessary and induced cytotoxic responses against all target cells tested, including target cells syngeneic to the responder cells. This activation was neither due to contaminating mitogen nor to the effect of heterologous serum in the assay system. The conclusion of these findings was that either polyclonal activation of CTL was induced by TCF1 or that some specific CTL clones differentiated into unrestricted killer cells under the influence of TCF1.

Introduction

Metabolically active antigen-presenting cells are required in order to stimulate both allo- (1, 2) and H-2-restricted (3, 4) T cell responses. Production of IL 1 (5) and subsequently of IL 2 (4, 6) are essential for the antigen-independent proliferation of IL 2 reactive Lyt-2$^+$ CTL precursor cells (7). IL 2 reactivity has been shown to be induced by a short exposure to specific ligands including allo-H-2 antigens (8). The process of this induction requires metabolic activity and active response of resting T cells (8).

A number of recent reports described the involvement of soluble factors other than IL 2 in the induction of CTL responses (9–16). The question

Abbreviations: *Con A* = Concanavalin A; *IL 1* = interleukin 1; *IL 2* = interleukin 2; *IFN-γ* = interferon-gamma; *TNP* = trinitrophenyl; *CTL* = cytotoxic T lymphocyte; *TCF1* = T cell cytotoxicity-inducing factor 1; *SR* = spontaneous release

whether these factors are involved in IL 2 responsiveness or at later stages of CTL maturation is unclear.

In this study, we report on the loss of antigen-specificity of the cytotoxic response when thymic responder cells were supplied with all required helper activities. Recently, we described the requirement for at least two helper factors, other than IL 2, for the differentiation of thymic precursor cells into cytotoxic effector cells (14, 17). One helper activity was required in the early phase of culture and was named T cell cytotoxicity-inducing factor 1 (TCF1). This activity was shown to be different from interleukin 1 (IL 1), IFN-γ, and colony stimulating factor (CSF). The requirement for the late-acting helper activity (T cell cytotoxicity inducing factor 2) was no longer seen when large amounts of semipurified IL 2 or recombinant IL 2 (> 40 U/ml) were added to the thymocyte cultures. The data presented here show that the presence of stimulator cells was no longer required when preparations containing both IL 2 and TCF1 were added to the cultures and that the resulting cytotoxic response was nonspecific.

Materials and Methods

Mice

C3H/TIF and DBA/2J were purchased from Bomholtgard, Ry, Denmark. C57BL/6 and Balb/c were bred in our own colony. All animals used were between 1 and 3 months of age.

Culture medium

Unless otherwise stated, the culture medium used was RPMI 1640 (GIBCO, powdered media) with L-Glutamine (2×10^{-3}M) and supplemented with 10 % heat-inactivated fetal calf serum (FCS), Hepes (0.02 M), 2-mercaptoethanol (4×10^{-5} M) and gentamicin (50 μg/ml). The protein-free culture medium used in some experiments is exactly as described by PECK and BACH (18). This medium was supplemented by 0.6 % heat-inactivated autologous mouse serum.

Lymphokine preparations

Concanavalin A- (Con A, Pharmacia) induced supernatants were prepared by conventional methods. Spleen cell suspensions were adjusted to 10^7 cells/ml in culture medium and incubated for 24 h with 5 μg Con A/ml in humidified atmosphere containing 5 % CO_2. Lymphokine preparations were stored at 4 °C.

Lymphokine preparations prepared by pulsing with Con A

Spleen cells (10^7/ml) were incubated in culture medium containing 10 μg Con A/ml. After 5 to 6 h the cells were washed 3 times with culture medium. Cells were readjusted to 10^7 cells/ml and cultured in Con A-free culture medium for 24 h at 37 °C. The maximal amount of Con A remaining in the supernatant was less than 0.01 μg/ml as determined by using trace-labelled Con A.

Absorption of IL 2

Con A-induced supernatants were depleted of IL 2 by absorption with an IL 2 receptor-bearing T cell line. Approximately 10^5 W-2 cells (14) were added to 1 ml of Con A supernatant

and incubated for 48 h. The cell-free supernatant was dialysed against culture medium and then incubated again for 48 h with 10^5 W-2 cells per ml. The supernatant was again dialysed against culture medium. IL 2 activity was less than 0.01 U/ml.

Preparation of partially purified IL 2

IL 2 was prepared as described by HILFIKER et al. (19). EL-4 thymoma cells were suspended at 10^6/ml in RPMI 1640 containing 0.5 % heat-inactivated FCS. Ten nanograms per ml of phorbol myristic acetate (PMA) were added, and the cells were incubated for 48 h. The cell-free supernatant was concentrated on an Amicon PM10 membrane. Proteins were precipitated with ammonium sulphate (80 % saturation). The precipitate was washed once and dissolved in water. After equilibration with 0.8 M ammonium sulphate in 0.01 M phosphate buffer, the sample was applied to a Phenyl-Sepharose column (Pharmacia). The column was washed with 2 volumes of 0.64 M ammonium sulphate containing 10 % ethanediol. The IL 2-containing peak was finally eluted with 40 % ethanediol in 0.16 M ammonium sulphate, concentrated on an Amicon PM10 membrane and equilibrated with column buffer (0.05 M Tris-hydroxymethylacetate, 0.1 M sodium chloride, pH 7.2, containing 0.05 % polyethylene glycol 6000). The material was then applied to a Sephadex G 100 column. IL 2 activity eluted with an apparent molecular weight of approximately 32,000. Peak fractions were pooled and dialysed against culture medium after adding FCS to a final concentration of 10 %.

Assay for IL 2 activity

The amount of IL 2 in supernatants was measured by their ability to support the growth of the IL 2-dependent cell line W-2 (14). Thymidine incorporation after a 20-h incubation and a 4-h pulse with 1.0 μCi of methyl-(^{3}H)-thymidine (^{3}(H)-TdR, specific activity 50 Ci/mmol, Amersham International Ltd, Amersham, UK) was determined. Activity is expressed in units used by FARRAR et al. (6).

T cell cytotoxicity assay

Thymic responder cells or nylon wool-purified spleen cells were cocultured in a final volume of 0.16 ml containing varying amounts of lymphokine preparations. Stimulator cells were 3×10^5 glutaraldehyde-fixed or X-irradiated (1500 R) allogeneic or TNP-haptenated syngeneic spleen cells, unless indicated otherwise. Cytotoxic activity was measured on day 5 in a 4-h ^{51}Cr-release test using either 5×10^3 labelled tumor targets (L929 mouse fibrosarcoma) or 2×10^4 (unmodified or TNP-modified) Con A-induced spleen cell blasts. Data are presented as specific ^{51}Cr-release (experimental count – spontaneous release/SDS count – spontaneous release) × 100. Spontaneous release (SR) was determined in supernatants of wells without added lymphokines. Data are means of triplicate cultures. The standard error was always less than 2.5 %.

Nylon wool treatment

Splenic responder cells were purified by one passage through a nylon wool column, according to JULIUS et al. (20).

Results

Induction of alloantigen-specific CTL responses in the presence of IL 2

Varying numbers of thymic responder cells were cultured for 5 days with X-irradiated allogeneic splenic stimulator cells in microcultures. The CTL response was measured by offering either syngeneic or allogeneic Con A blasts targets with the stimulator cells. As shown in Figure 1, no lytic

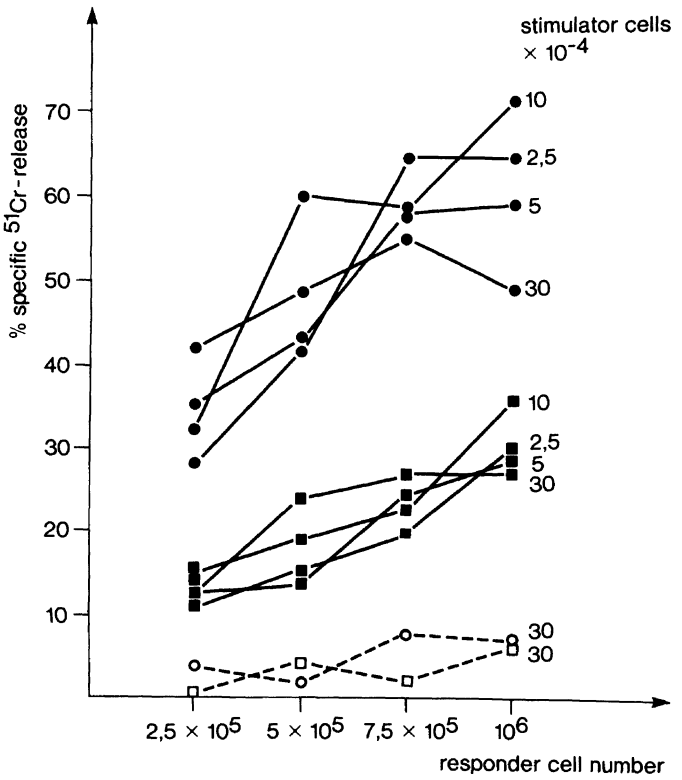

Fig. 1. Varying numbers of C57BL/6 thymic responder cells were incubated with varying numbers of gamma-irradiated (1500 R) splenic stimulator cells of either C3H (●) or Balb/c (■) mice. 10 µl of IL 2 derived from EL-4 supernatant (800 U/ml IL 2) was added to the cultures. The data represent %specific ^{51}Cr-release measured on 2×10^4 prelabelled C3H targets. Without the addition of helper factors, less than 10% specific ^{51}Cr-release was measured at all responder or stimulator doses (open symbols). When Balb/c targets were used, corresponding specificity was obtained.

activity was seen in the absence of added helper factors. However, in the presence of IL 2, cytotoxicity was observed for both targets. In both cases, the response was not dependent upon the number of stimulator cells used in the test. The cytotoxic response measured on targets syngeneic with the stimulators was at all responder numbers significantly higher than the cross-reactivity measured on inappropriate targets. This observation was confirmed in experiments with different strain combinations.

Table 1 shows the degree of specificity and cross-reactivity in the presence of IL 2 in a representative experiment. In all experiments where metabolically active stimulator cells were used, preferential lysis of the sensitizing alloantigen was observed. Taking the respective lysis of stimulator-syngeneic target cells as 100%, the lysis of the H-2-different target cells was 47% with DBA targets and 61% with C57BL/6 targets.

Similar data were obtained with other responder cells. Little cytolytic activity (< 10 %) was induced by IL 2 preparations alone in the absence of stimulator cells.

Table 1. Stimulator-dependent cytotoxic responses in presence of IL 2

Responder[a]	Stimulator	IL 2[b]	% specific ^{51}Cr-release[c]	
			Target:	
			DBA	C57BL/6
C3H	–	–	0.0	0.1
C3H	–	+	0.0	9.2
C3H	C57BL/6	–	0.0	0.0
C3H	C57BL/6	+	14.3	57.6
C3H	DBA	–	0.0	0.0
C3H	DBA	+	30.3	35.4

[a] Five × 10^5 thymic responder cells were incubated with 3 × 10^5 of the indicated gamma-irradiated (1.500 rad) stimulator cells.
[b] Ten µl of semipurified IL 2 (800 U/ml IL 2) was added to some of the cultures.
[c] After 5 days of culture % specific ^{51}Cr-release was measured in a 4-h release test with 2 × 10^4 prelabelled target cells. Experiments with C57BL/6 responder cells and C3H or DBA stimulator cells have also been performed and gave corresponding results.

Table 2. Effect of stimulator quality on cytotoxic responses in the presence of different lymphokines

Stimulator[a]	Lymphokines[b]	% specific ^{51}Cr-release[c]		
			Target:	
		C3H	C57BL/6	Balb/c
C3H gamma-irradiated	–	0.0	0.0	0.0
C3H gamma-irradiated	IL 2	62.8	38.5	19.0
C3H gamma-irradiated	IL 2 + TCF1	66.1	48.4	22.4
C3H gamma-irradiated	Con A lymphokine	54.3	30.3	12.9
C3H glutaraldehyde-fixed	–	4.1	0.0	1.1
C3H glutaraldehyde-fixed	IL 2	13.6	4.7	9.3
C3H glutaraldehyde-fixed	IL 2 + TCF1	44.3	18.5	20.3
C3H glutaraldehyde-fixed	Con A lymphokine	32.7	13.1	8.3
–	–	0.0	0.0	0.0
–	IL 2	3.9	1.4	3.0
–	IL 2 + TCF1	45.4	25.0	24.2
–	Con A lymphokine	42.2	28.4	20.2

[a] Two × 10^5 Balb/c thymic responder cells were incubated with 3 × 10^5 of the indicated stimulator cells for 5 days.
[b] The following lymphokine preparations were added to the cultures: IL 2, semipurified from EL-4 supernatant (800 U/ml IL 2), 20 µl; TCF1, IL 2-absorbed C3H Con A lymphokine (< 0.01 U/ml IL 2), 60 µl; C3H Con A lymphokine (79 U/ml IL 2) 60 µl.
[c] The data represent % specific ^{51}Cr-release from 2 × 10^4 prelabelled target cells. The TCF1 preparation alone had no activating effect.

Table 3. Induction of cytotoxic responses with TCF1 and recombinant IL 2[a]

Lymphokines[b]	% specific ^{51}Cr-release[c]
–	0.2
Con A lymphokine	65.0
TCF1	0.5
IL 2	5.5
Recombinant IL 2	3.2
IL 2 + TCF1	80.8
Recombinant IL 2 + TCF1	69.8

[a] One × 10^5 C3H thymic responder cells were incubated with 3 × 10^5 glutaraldehyde-fixed TNP-modified syngeneic stimulator cells.

[b] The following lymphokine preparations were added to the cultures: C3H Con A lymphokine (79 U/ml IL 2), 60 µl; TCF1, IL 2-absorbed C3H Con A-lymphokine (< 0.01 U/ml IL 2), 60 µl; IL 2, semipurified from EL_4 supernatant (800 U/ml IL 2), 20 µl; recombinant IL 2 (800 U/ml IL 2), 20 µl.

[c] The data represent % specific ^{51}Cr-release from 5 × 10^3 prelabelled TNP-modified L929 target cells.

Replacement of stimulator cells by TCF1

The data in Table 2 demonstrate that the requirement for TCF1 in addition to IL 2 was dependent on the quality of the stimulator cells. Thymic responder cells could be induced for cytotoxicity with irradiated splenic stimulator cells, provided the cultures contained IL 2. About the same level of cytotoxicity was measured when either a combination of IL 2 and TCF1, or Con A-induced supernatant was added. The same responder population developed little or no cytotoxic activity with metabolically inactive stimulators or without any stimulator cells, although the same amount of IL 2 was present. The cytotoxic responses developed with TCF1 and IL 2 were not specific for the stimulating antigen. Irrelevant and even syngeneic targets were lysed under these conditions. TCF1 alone had no activating effect in any of the cultures.

Table 3 demonstrates that the semipurified IL 2 used in all the experiments could be replaced by recombinant IL 2 at the same concentration without changing the cytotoxic responses obtained. Cultures containing metabolically inactive stimulator cells and 80 U IL 2/ml were only dependent on the addition of TCF1 preparations for induction of cytotoxicity. Even very much higher concentrations of IL 2 (up to 1000 U per ml) did not eliminate the requirement for TCF1 (data not shown).

The data in Figure 1, Table 1, and Table 2 suggest that the cytotoxicity induced in cultures of X-irradiated stimulator cells and thymic responder cells may be due to endogenous TCF1. TCF1 itself confers no antigen specificity, as no differences were observed when TCF1 preparations from different mouse strains were tested (Table 4). Nonspecific activation by residual Con A was excluded since supernatants from Con A-pulsed spleen cells were used as a source of TCF1. In these supernatants, less than 0.01 µg

Table 4. Cytotoxic responses in the presence of IL 2 and TCF1 from Con A-pulsed lymphokines

Responder[a]	Stimulator	IL 2[b]	TCF1[b]	% specific ^{51}Cr-release Target : C3H
Balb/c	C3H gamma-irradiated	–	–	1.9
Balb/c	C3H gamma-irradiated	+	–	37.9
Balb/c	C3H gamma-irradiated	+	DBA	59.0
Balb/c	C3H gamma-irradiated	+	C3H	55.1
Balb/c	–	–	–	5.4
Balb/c	–	+	–	7.5
Balb/c	–	+	DBA	41.2
Balb/c	–	+	C3H	20.9
C57BL/6	C3H gamma-irradiated	–	–	6.4
C57BL/6	C3H gamma-irradiated	+	–	59.9
C57BL/6	C3H gamma-irradiated	+	DBA	61.6
C57BL/6	C3H gamma-irradiated	+	C3H	59.6
C57BL/6	–	–	–	8.6
C57BL/6	–	+	–	9.4
C57BL/6	–	+	DBA	22.7
C57BL/6	–	+	C3H	19.9

[a] Two × 10^5 thymic cells of the indicated strain were incubated with or without 3 × 10^5 stimulator cells.

[b] The following lymphokine preparations were added to the cultures: IL 2, semipurified EL-4 supernatant (800 U/ml IL 2) 20 µl; TCF1, Con A-pulsed lymphokine from either DBA spleen cells (9.5 U/ml IL 2) or C3H spleen cells (26 U/ml IL 2), 20 µl.

[c] The data represent %specific ^{51}Cr-release from 2 × 10^4 prelabelled C3H target cells.

Con A/ml was detectable using trace labelled Con A. Such concentrations of Con A did not cause polyclonal activation in our assay system (data not shown). Comparable amounts of semipurified IFN-γ and IL 1 were unable to substitute for TCF1 (14, 17).

Induction of cytotoxic activity against syngeneic targets in the presence of helper factors

C3H (H-2^k) thymocytes or nylon wool-purified spleen cells were stimulated with IL 2 and TCF1 (Table 5) and tested on L929 syngeneic targets. Again, polyclonal activation by remaining Con A in the TCF1 preparation was excluded by utilizing a mitogen-pulsed spleen cell supernatant with less than 0.01 µg Con A/ml.

Induction of cytotoxic responses in cultures supplemented with autologous serum

The same experiments as described above were carried out in medium containing autologous mouse serum instead of fetal calf serum. The purpose of these experiments was to test whether the FCS in the cultures could be

Table 5. Cytotoxic activity against syngeneic targets in the presence of IL 2 and TCF1

Responder[a]	IL 2[b]	TCF1[b]	% specific ^{51}Cr-release[c] Target: L929
A) C3H thymocytes	−	−	1.2
	+	−	0.0
	−	+	0.0
	+	+	53.8
B) C3H spleen cells	−	−	1.3
	+	−	7.3
	−	+	0.6
	+	+	35.7
C) C3H spleen cells	−	−	4.9
	+	−	10.2
	−	+	0.0
	+	+	32.4

[a] One × 10^5 thymic cells or 1 × 10^5 2-times nylon wool-purified splenic cells were used as responder cell population and cultured without stimulator cells.

[b] The following lymphokine preparations were added to the cultures: IL 2, semipurified EL-4 supernatant (800 U/ml IL 2) 20 µl; TCF1 in A and B, IL 2-absorbed C3H Con A lymphokine (< 0.1 U/ml IL 2), 40 µl; TCF1 in C, Con A-pulsed C3H lymphokine (26 U/ml IL 2), 60 µl.

[c] The data represent % specific ^{51}Cr-release from 5 × 10^3 prelabelled L929 target cells.

the reason for the nonspecific cytotoxic responses induced with TCF1 plus IL 2. FCS has been shown not only to provide antigenic determinants recognized by T cells (21) but also to stimulate helper T cell activity (22) and polyclonal T cell responses (23).

Table 6. Cytotoxic responses induced with IL 2 and TCF1 in cultures supplemented with autologous serum

IL 2[a]	TCF1[a]	% specific ^{51}Cr-release[b]		
			Target:	
		TNP-L929	C3H	C57BL/6
−	−	0.0 ± 0.5	3.1 ± 2.3	1.3 ± 0.9
+	−	0.0 ± 0.3	0.8 ± 1.5	1.5 ± 2.6
−	+	0.2 ± 0.7	3.1 ± 2.6	2.6 ± 0.7
+	+	12.8 ± 2.6	12.4 ± 3.4	8.1 ± 0.7

[a] Two × 10^5 C3H thymus cells were incubated with 3 × 10^5 UV-irradiated TNP modified syngeneic stimulator cells. The following lymphokine preparations were generated in FCS-free culture medium containing 0.6 % heat-inactivated autologous mouse serum: IL 2, semipurified EL-4 supernatant (1.450 U/ml IL 2) 20 µl; TCF1, Con A-pulsed C3H lymphokine (54 U/ml IL 2) 80 µl.

[b] The data represent % specific ^{51}Cr-release ± SD from 5 × 10^3 prelabelled TNP-L929 target cells or 2 × 10^4 prelabelled blast cells as targets.

Table 7. Inhibition of lymphokine-induced cytotoxic responses by cold targets[a]

	% specific ^{51}Cr-release[c] Targets							
	C57BL/6				Balb/c			
Cold targets[b] ($\times 10^{-4}$)	0.5	2	8	32	0.5	2	8	32
C57BL/6	17.6	9.7	10.4	0.2	35.8	23.3	27.9	14.3
C3H	19.3	16.0	11.4	8.8	33.1	18.8	17.9	10.2
Balb/c	22.4	17.0	12.8	7.9	36.0	19.9	18.5	7.1
None			23.2				35.7	

[a] One × 10^5 C3H thymic responder cells were incubated with 3 × 10^5 glutaraldehyde-fixed C57BL/6 splenic stimulator cells for 5 days in the presence of TCF1 (8U/ml IL 2), 80 µl and IL 2 (50 U/ml IL 2), 20 µl.

[b] The cold targets were not labelled with ^{51}Cr but were otherwise prepared according to the same procedure as the labelled cells.

[c] The data represent % specific ^{51}Cr-release from 2 × 10^4 labelled target cells.

The data given in Table 6 demonstrate induction of cytotoxic responses with TCF1 plus IL 2 in FCS-free cultures. For these experiments, all lymphokine preparations were also generated under FCS-free conditions. Again, target cells syngeneic to the responder population were lysed when optimal concentrations of TCF1 and IL 2 were present.

Inhibition of cytotoxic responses by the addition of cold targets

Cold target inhibition studies were done to test whether the cytotoxic effector cells induced by addition of sufficient amounts of IL 2 and TCF1 were specific for a certain target or were nonrestricted. As shown in Table 7, the cytotoxic response induced in C3H thymic responder cells in the presence of metabolically inactive C57BL/6 stimulator cells was directed against both C57BL/6 and Balb/c targets. The lysis of both targets was inhibited with cold targets of either C57BL/6, Balb/c or C3H origin. This indicated that the cytotoxic effectors were antigen-unspecific.

Discussion

The experiments in this report show that cytotoxic effector cells develop from thymocytes in the absence of stimulator cells, provided sufficient helper factors are added to the cultures. This effect was not due to contaminating mitogen in the TCF1 preparations. In earlier reports, we described that the effect of TCF1 was not due to other known lymphokine activities such as IL 1, IFN-γ or CSF (14, 17).

Specificity and requirement for the sensitizing antigen were obvious as long as X-irradiated allogeneic stimulator cells and IL 2 were included in

the thymocyte cultures. When stimulator cells were replaced by TCF1, all offered target cells were lysed. There are several possible explanations for this phenomenon: 1) Antigen contact is not essential for activation of the CTL precursor cells when all necessary helper factors are provided (polyclonal activation). 2) Some specific CTL clones acquire unrestricted cytotoxic potential in the presence of TCF1. 3) *In vitro* cultures contain a whole spectrum of artificial antigens, such as FCS. 4) Some *in vivo* primed CTL clones are expanded *in vitro* and crossreact with the targets offered. Cytotoxic responses generated in the absence of FCS (Table 6) indicate, however, that heterologous serum does not explain the antigen-nonspecific cytolytic responses induced by soluble mediators. The relatively weak cytotoxic responses measured in the absence of FCS are to be expected if one considers FCS to exert an enhancing effect (21–23). The cold target inhibition experiment shown in Table 7 suggests that the cytotoxic cells induced by TCF1 are antigen unspecific since their cytotoxic response was inhibited by all target cells. A number of recent reports describe the development of natural killer-like unrestricted cytotoxicity from CTL when lymphokines were provided in large amounts (24–26) or when the cells were cultured for a longer period in the presence of metabolically active stimulator cells (27, 28). Preliminary phenotypic characterization of the effector cells in our 5-day lymphokine-induced thymocyte cultures showed that they were sensitive to antibodies against Lyt-2^+ and complement. About 80 % of the effector cells were positive for Thy-1 and Lyt-2 as shown by fluorescence-activated cell sorting analysis, thus carrying surface markers of CTL. In this respect, these cells resemble the lymphokine-activated, antigen-independent CTLs described by BROOKS et al. (29), and their cytotoxicity resembles the «lymphokine-activated killer cell phenomenon» described by several laboratories (30, 31).

The biological effects of TCF1 described here are similar to those effects ascribed to the allogeneic effect factor (AEF) reviewed by ALTMAN and KATZ (32). AEF is reported to augment weak cytotoxic T cell responses and to induce autonomous cytotoxicity, i.e. cytotoxicity in the absence of stimulator cells. It has been shown that AEF preparations induced cytotoxic responses against self and that such responses were not FCS-related artefacts. Thus, we believe that we are possibly studying the same mechanism of T cell activation but are using helper mediators from different sources. Also, the availability of semipurified and recombinant IL 2 (Table 3) enables to distinguish between the effect of TCF1 and IL 2 itself.

Recent results from our laboratory indicate that TCF1 is produced by T helper cells (unpublished results). Thus, it appears that activated T helper cells as stimulators generate all lymphokines necessary to produce CTL from precursor cells. Recent reports indicate that activated helper T cells are indeed good stimulator cells for the induction of primary CTL responses (33, 34). Specificity of the CTL response in this concept is achieved by the support of only those CTL precursor cells that are in close contact to the

activated helper T cell, due to their receptor for the antigenic determinant on the helper cell.

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Recombinant human IL 2 (2×10^5 U/ml) was obtained from CETUS Corporation. The material was 98 % pure and contained 0.02 ng/ml endotoxin.

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