Tissue Destruction Resulting from the Interaction of Cytotoxic T Cells and Their Targets[a]

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The work reviewed here began as an attempt to examine the *in vivo* relevance of a group of cytotoxic T lymphocyte (CTL) clones directed against the then newly described histocompatibility (H) antigen, epidermal alloantigen-1 (Epa-1).[1] Because, as its name implies, Epa-1 is preferentially expressed on epidermal cells (EC), as determined in cell-mediated cytotoxicity assays *in vitro*,[2] we were particularly interested in determining whether Epa-1–specific CTL would attack allogeneic skin cells *in vivo*. Thus, we were gratified to find that relatively small numbers of clone 21-4, one of our most reliable Epa-1–specific clones, evoked full-thickness skin necrosis in an immunologically specific, major histocompatibility complex (MHC)–restricted, dose-dependent fashion upon intradermal inoculation into appropriate allogeneic hosts.[3,4]

The necrotic skin lesions that we initially evoked with clone 21-4,[4] and subsequently with several other Epa-1–specific CTL clones generated entirely *in vivo*,[5] represent an intense form of the "immune lymphocyte transfer reaction." This reaction was first described in 1958 by Brent *et al.*[6] in the guinea pig and then

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subsequently described by them[7] and others[8,9] in the rabbit. Billingham and Streilein's laboratory described this reaction in the hamster,[10-12] rat,[13] mouse,[14,15] and dog.[16] At the time of these reports, the effector cells for transfer reactions were crude suspensions of lymph node cells (LNC) and spleen cells (SC) from animals sensitized with a skin allograft, without any subsequent *in vitro* manipulation to boost alloimmunity. Consequently, reactions elicited with such cells rarely progressed beyond induration. By contrast, in our studies the lesions progressed to full-thickness necrosis. To determine whether this intense reaction reflected our use of cloned or *in vitro*-selected CTL, we tested the ability of cells sensitized in a classic or contemporary fashion to evoke transfer reactions in parallel in two inbred mouse strain combinations. For the classic test, we immunized hosts with

TABLE 1. Classic versus Contemporary Immune Lymphocyte Transfer Reactions

	Reactions Grades[a]										
	C3H/He Anti-CBA				C3H/He Anti-BALB/c						
Day	LNC[b]		CTL[c]		LNC			CTL			
1	1+	1+	3+	3+	1+[d]	1+	1+	3+[d]	3+	3+	3+
2	2+	3+	3+	3+	2+	2+	2+	4+	3+	3+	5+
3	2+	4+	4+	5+	2+	1+	2+	5+	4+	4+	5+
4	2+	4+	5+	5+	1+	1+	1+	5+	4+	5+	5+
5	2+	4+	5+	5+	0	0	0	5+	5+	5+	5+
6	1+	5+	5+	5+	0	0	0	5+	5+	5+	5+
7	4+	5+	5+	5+							
8	5+	5+	5+	5+							
CMC[e]	0		61%		0			91%			

[a] 0, no perceptible response; 1+, barely perceptible swelling; 2+, swelling 3–4 mm in diameter, site soft; 3+, swelling ≥5 mm in diameter, site firm; 4+, large reaction with indurated core; 5+, site ulcerated or necrotic. Grades shown are the highest of three injection sites per host, except for the two hosts that received 2.5×10^7 cells at a single site. Grading was performed daily 1–8 days after injection of LNC or CTL.

[b] Draining lymph node cells (LNC) from skin-grafted hosts (classic test).

[c] *In vitro*-generated cytotoxic T lymphocytes (contemporary test).

[d] These two hosts received 2.5×10^7 cells; all others, 1×10^7 cells per site.

[e] Percent specific lysis of host-strain splenic lymphoblasts by effector LNC or CTL in 3 hr chromium-release cytotoxicity assays at an effector-to-target-cell ratio of 25 : 1.

a single skin allograft and harvested LNC from draining lymph nodes;[14] for the contemporary test, we primed hosts with an intraperitoneal inoculation of 1×10^7 allogeneic SC and then generated CTL from host SC *in vitro* in one-way mixed lymphocyte cultures.[2]

As seen in TABLE 1, the CTL evoked earlier and more intense transfer reactions than the LNC in both the H-2–compatible C3H/He anti-CBA and the H-2–incompatible C3H/He anti-BALB/c strain combinations. The differences in the effectiveness of the CTL and LNC were particularly apparent in the latter combination, where the reactions evoked by the LNC never even became indurated, whereas all the reactions evoked by the CTL developed ulceration. In fact, we were surprised to find that the reactions induced by LNC in the C3H/He anti-CBA strain combination eventually ulcerated, inasmuch as Streilein *et al.*[13-15] did

not observe reactions of this intensity in their original descriptions of murine transfer reactions. We used LNC obtained exclusively, however, from lymph nodes, draining the site of the sensitizing skin allograft, whereas Streilein *et al.* pooled LNC from draining and contralateral nodes (J. W. Streilein, personal communication). Regardless, our observations on the ability of allospecific CTL—particularly those cloned from rejecting allografts and draining lymph nodes[5]—to induce ulcerative transfer reactions demonstrate that allogeneic tissue can be destroyed through the direct mediation of CTL. This finding has clear relevance to the question of which T cells mediate allograft rejection.[17,18]

More recently, we discovered that in certain contexts CTL also can mediate destruction of syngeneic tissue,[19] apparently by initiating events that lead to an intense inflammatory reaction on the part of the host itself. This observation also has a precedent in the early work of Brent *et al.*[7] They were interested in establishing that transfer reactions were provoked "by a local engagement of sensitized cells with antigen." To do this, they used normal hosts as "neutral soil for the interaction of antigen with sensitized cells," a principle previously established by those investigating the tuberculin reaction in the guinea pig.[7] Thus, Brent *et al.* mixed sensitized A anti-*B* lymphoid cells (LC) with B cells and injected the mixtures into A hosts—hosts syngeneic to the effector cells; they reported feeble though significant cutaneous inflammatory reactions. Stronger reactions of this nature were described by Ramseier and Streilein,[11] who injected mixtures of sensitized host-strain LC and allogeneic LC or EC into the skin of irradiated hamsters. Once more, however, the reactions were never scored as necrotic, again presumably reflecting the use of unselected effector cell populations with a low frequency of specifically sensitized CTL.

We first evoked "innocent bystander" reactions with CTL directed against Epa-1, the previously mentioned tissue-restricted, non-H-2 H antigen, well expressed on EC, fibroblasts, and activated macrophages, but poorly expressed, if at all, on LC.[20] Mixtures of Epa-1–specific bulk-culture or cloned CTL of strain C3H/He origin and Epa-1$^+$ strain CBA EC evoked grossly observable skin ulceration 3–5 days after injection into the skin of syngeneic C3H/He hosts.[19] As seen in FIGURE 1, as few as 5×10^6 cloned CTL, mixed with an equal number of allogeneic EC, evokes an intense inflammatory reaction, with tissue necrosis extending from the panniculus carnosus to the epidermal surface. Clone NR46, which produced the lesion shown in the FIGURE, was derived from CTL generated entirely *in vivo* in EC-impregnated sponge-matrix allografts.[5] These Epa-1–specific clones are Lyt-2$^+$ and L3T4$^-$, as determined by flow cytometry[5]—they express the classic phenotype of MHC class I–directed mouse CTL—and they lyse EC *in vitro* in an antigen-specific, H-2–restricted, dose-dependent fashion.[4,5]

The transfer and bystander reactions in the Epa-1 and other CTL–target-cell systems we have studied to date have virtually the same kinetics. But as seen in FIGURE 2, the latter reactions are not as consistent as the former. For example, all of the Epa-1 transfer reactions ulcerated compared to 82% of the bystander reactions. Moreover, in contrast to transfer-reaction ulcers, which often exceed 5 mm in diameter, bystander-reaction ulcers are rarely more than 2 mm across, though the swelling and induration at the site often exceed 10 mm. Nevertheless, the degree of tissue necrosis seen in bystander-reaction lesions (FIG. 1) is remarkable considering that the only source of specific antigen for the CTL are several million admixed target cells. Both transfer and bystander reactions are self-limiting, and the incidence of ulcerative lesions usually does not increase after five days. In the usual experiment, the ulcers heal within ten days after injection. Ulcers, however, may persist up to 15 days if the hosts are supplied with exogenous T-cell growth

FIGURE 1. C3H/He mouse skin at the site of an intradermal injection three days earlier of 5×10^6 C3H/He clone NR46 cytotoxic T lymphocytes (CTL) together with 5×10^6 CBA epidermal cells (1 μm, Epon-embedded, Giemsa-stained sections). **a:** The solid arrow indicates the border between viable epidermis (extending to the right of the arrow) and necrotic epidermis (extending to the left of the arrow). The open arrowhead indicates a hair follicle within the region of necrotic dermis. Two viable hair follicles within the area of unaffected dermis are indicated by solid arrowheads (×100). **b:** Site of injection of clone NR46 CTL and keratinocytes (some of the latter indicated by solid arrowheads) in the deep dermis. There is extensive inflammation. In addition, a skeletal muscle fiber of the panniculus carnosus exhibits focal necrosis (open arrowhead) as well as a region that appears viable (solid arrow) (×250).

factor in the form of interleukin-2 (IL-2)–rich culture supernatants (data now shown). The dependence of the reactions on IL-2 is also evident from the finding that 1×10^6 Epa-1–specific CTL, which normally are too few to evoke ulcerative transfer reactions, do so when they are injected suspended in IL-2–rich supernatant fluid instead of conventional medium (data not shown).

FIGURE 2 also illustrates the specificity of the transfer and bystander reactions. Epa-1–specific CTL produce ulcerative transfer reactions in allogeneic CBA but

not in syngeneic C3H/He hosts, and the same CTL evoke ulcerative bystander reactions when admixed with allogeneic CBA EC but not syngeneic C3H/He EC. CBA EC targets alone evoked grade 2+ and 3+ reactions that were most intense during the first day after injection. These transient reactions probably resulted from inflammation caused by stratum corneum antigens,[21] because reactions of the same intensity were evoked by syngeneic C3H/He EC, and reactions evoked by injections of CBA LC alone were barely perceptible (data not shown).

Although we first described ulcerative transfer reactions with Epa-1 CTL,[3-5] they are by no means limited to this H-antigen system, but are evoked almost invariably by CTL directed against a variety of non-H-2 and H-2 antigens, as seen in TABLE 2. Surprisingly, bystander reactions with alloreactive CTL are evoked much more readily against non-H-2 than against H-2 antigens: we evoked ulcerative bystander lesions in only one of ten H-2 incompatible compared to four of five H-2-compatible strain combinations. The lack of bystander reactivity of most of

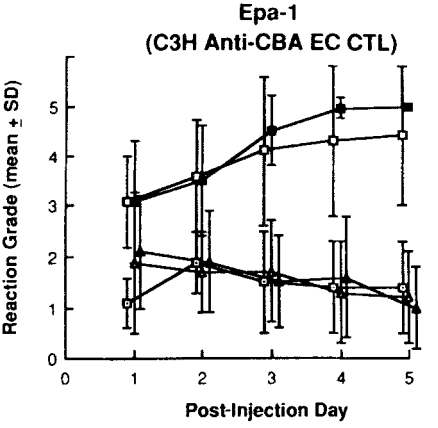

FIGURE 2. Kinetics of immune lymphocyte transfer and bystander reactions in the Epa-1 histocompatibility system. For reaction grades, see TABLE 1. CTL (C3H/He anti-CBA epidermal cells, EC) to CBA hosts (-■-, n = 26); CTL to C3H/He hosts (-□-, n = 26); CTL plus CBA EC to C3H/He hosts (-□-, n = 33); CTL plus C3H/He EC to C3H/He hosts (-▲-, n = 8); CBA EC alone to C3H/He hosts (-△-, n = 12).

the H-2-specific CTL was unexpected given that these same CTL invariably produced H-2-specific ulcerative transfer reactions. The defect does not seem to be due to an H-2 Ir gene effect,[22] because the incidence of ulcerative bystander lesions evoked by DBA/2 and BALB/c CTL—both H-2^d—directed against the same C57BL/6 targets was 64% and 0%, respectively. Nor can it simply be due to non-H-2 Ir gene effects:[22] the incidence of ulcerative bystander lesions evoked by DBA/2 CTL directed against C57BL/6, BALB/c, and CBA targets was 64%, 23%, and 0%, respectively (TABLE 2).

We think that mechanisms similar to those activated during intense delayed-type hypersensitivity (DTH) reactions may be responsible for the tissue destruction seen in bystander reactions (see below). If this is so, then the difficulty in evoking ulcerative bystander lesions with H-2-specific CTL may reflect the well-established, though not well-appreciated, fact that non-H-2 antigens are actually

TABLE 2. Incidence of Ulcerative Skin Lesions Evoked by Bulk-Culture CTL in Immune Lymphocyte Transfer and Bystander Reactions in Various Inbred Mouse Strain Combinations

Target Antigen(s)	Strain Combination	H-2 Haplotype	Transfer Reactions[a]		Bystander Reactions[b]	
	Donor Anti-Host	Donor/Host	No./No. Tested	Percent	No./No. Tested	Percent
Epa-1	C3H/He anti-CBA[c]	k/k	26/26	100	27/33	82
H-Y	C57BL/6 female anti-C57BL/6 male	b/b	8/9	89	7/11	64
Multiple	C3H/He anti-CBA	k/k	9/10	90	12/16	75
Non-H-2	CBA anti-C3H/He	k/k	9/9	100	2/6	33
	DBA/2 anti-BALB/c	d/d	8/9	89	3/13	23
	BALB/c anti-DBA/2	d/d	4/4	100	0/4	0
H-2	C57BL/6 anti-A/J	b/a	7/7	100	0/8	0
	C57BL/6 anti-BALB/c	b/d	5/5	100	0/10	0
	C57BL/6 anti-DBA/2	b/d	4/4	100	0/4	0
	C57BL/6 anti-CBA	b/k	2/2	100	0/7	0
	BALB/c anti-C57BL/6	d/b	7/7	100	0/12	0
	DBA/2 anti-C57BL/6	d/b	11/11	100	9/14	64
	DBA/2 anti-CBA	d/k	4/4	100	0/4	0
	CBA anti-C57BL/6	k/b	3/3	100	0/6	0
	C3H/He anti-C57BL/6	k/b	4/4	100	0/9	0
	C3H/He anti-BALB/c	k/d	9/9	100	0/4	0
TNP	CBA anti-TNP-CBA[d]	k/k	NA[e]		27/42	64
Influenza A	DBA/2 anti-flu-DBA/2[f]	d/d	NA		18/29	62

[a] Evoked with an intradermal inoculation of 25×10^6 CTL.
[b] Evoked with an intradermal inoculation of a mixture of 25×10^6 CTL and 5×10^6 epidermal or spleen cell targets.
[c] Epidermal cell immunogens and targets; the tests with all other strain combinations involved spleen cell immunogens and targets.
[d] 2,4,6-trinitrophenyl-conjugated syngeneic CBA spleen cells.
[e] Not applicable.
[f] Influenza A virus-infected syngeneic DBA/2 spleen cells.

more effective than H-2 antigens in evoking DTH.[23-27] To explain this, Ohori *et al.*[26] proposed that although H-2 antigens represent a stronger stimulus, they also may provoke "a negative regulatory response, which the minor antigens escape." This could be the induction of suppressor T cells (T_s). It is important to reemphasize, however, that the same H-2–specific CTL consistently evoke ulcerative transfer reactions in H-2–incompatible strain combinations where they fail to evoke bystander reactions. But, in contrast to the latter reactions, where the admixed target cells represent a very limited source of alloantigen, in transfer reactions, where CTL are injected directly into allogeneic skin, alloantigen essentially is unlimited. The latter situation may result in much less pervasive down-regulation by T_s. The hypothesis that T_s dominate bystander reactions in H-2 but not in non-H-2 antigen systems is testable, and experiments along these lines currently are in progress. For example, we are determining the effect of combining H-2 and non-H-2 CTL–target-cell mixtures in the same inoculum and whether T_s actually are present in strain combinations where CTL–target-cell mixtures fail to evoke bystander reactions.

The capacity to initiate ulcerative bystander reactions is not limited to alloreactive CTL but also is a characteristic of CTL directed against hapten-modified and virus-infected target cells in totally syngeneic systems. Our observations in these systems, fully described elsewhere,[28] are summarized at the bottom of TABLE 2. Mixtures of specifically sensitized CTL and trinitrophenyl-modified or influenza A virus-infected syngeneic SC evoked necrotic, ulcerative bystander lesions in the skin of syngeneic hosts with a similar incidence to those evoked by alloreactive CTL directed against non-H-2 antigens. Thus, bystander reactions are not peculiar to CTL specific for H antigens but appear to be a general manifestation of CTL–target-cell interactions in non-MHC systems.

A fundamental characteristic of the T-cell receptor is its ability to recognize antigen only when the antigen is associated on the cell surface with a molecule encoded by the MHC,[29] the H-2K^k gene product in the case of Epa-1.[30] Thus, we previously established that the capacity of both bulk-culture and cloned Epa-1–specific CTL to lyse EC *in vitro* and to evoke transfer reactions *in vivo* shows the identical H-2 restriction specificity.[3-5] For example, clone 21-4 CTL evoke ulcerative lesions in the skin of H-2K^k B10.A and B10.BR hosts but not in the skin of H-2K^b B10.MBR and H-2K^d B10.OL hosts,[4] even though all of these H-2 congenic and recombinant strains on the C57BL/10 background are Epa-1$^+$.[31] Therefore, we were fascinated to observe that the capacity of Epa-1–specific CTL to evoke ulcerative bystander lesions is apparently not H-2–restricted.[19] For example, mixtures of H-2^k C3H/He CTL and CBA EC evoke ulcerative lesions in Epa-1$^-$, H-2^b C3H.SW hosts as well as they do in C3H/He hosts, and C3H/He CTL are just as capable of evoking ulcerative bystander reactions in syngeneic hosts when they are injected together with Epa-1$^-$, H-2^b C57BL/6 EC as with Epa-1$^+$, H-2^k CBA EC.[28] In fact, as seen in TABLE 3, the current overall incidence of ulcerative bystander reactions evoked with H-2–incompatible and –compatible target cells and hosts is virtually identical, whereas there is a marked difference in the incidence of transfer reactions in H-2–compatible and –incompatible hosts in the Epa-1 system. The substitution of H-2–incompatible for H-2–compatible hapten-modified target cells did not significantly reduce the incidence of ulcerative bystander reactions in the trinitrophenyl (TNP) system, although the substitution of H-2–incompatible hosts did (TABLE 3). By contrast, although the data are incomplete, it appears that bystander reactions in the influenza A system do follow the rules of MHC restriction (TABLE 3).

Because the associative recognition of antigen with an MHC gene product is such an important feature of the T-cell receptor,[29] we are examining the apparent disregard for this in our bystander-reaction systems very critically. For example, the use of H-2–incompatible target cells and hosts in bystander-reaction systems runs the risk of stimulating local cutaneous host-versus-graft reactions against the foreign H-2 antigens expressed by the CTL or admixed target cells, and we have preliminary evidence that such reactions apparently can give rise to ulcerative skin lesions in certain strain combinations (data not shown). In addition to this relatively trivial explanation of the nonrestricted appearance of bystander reactions, we also are examining the possibility that H-2–compatible antigen-presenting cells (APC) in the host or in the cell inoculum might circumvent MHC restriction by presenting antigen shed from the admixed allogeneic target cells to the CTL. For example, when ulcerative skin lesions are evoked in C3H/He ($H\text{-}2^k$) hosts by mixing C57BL/6 ($H\text{-}2^b$) instead of CBA ($H\text{-}2^k$) EC with C3H/He anti-

TABLE 3. Major Histocompatibility Complex (H-2) Restriction Tests of Transfer and Bystander Reactions

Non-H-2 Antigen System	Reaction System	No. of Ulcerative Skin Lesions/No. Tested (Percent)		
		H-2–Compatible[a] Target Cells and Hosts	H-2–Incompatible[a]	
			Target Cells[b]	Hosts[c]
Epa-1	Transfer	26/26 (100)	NA[d]	2/27 (7)
	Bystander	27/33 (82)	10/14[e] (71)	12/15 (80)
TNP[f]	Bystander	27/42 (64)	6/12[g] (50)	3/13[h] (23)
Flu[i]	Bystander	18/29 (62)	3/13[j] (23)	0/3

[a] In relation to the injected bulk-culture cytotoxic T lymphocytes (CTL).
[b] Hosts H-2–compatible in relation to the CTL.
[c] Admixed target cells (in bystander reactions) H-2–compatible in relation to the CTL.
[d] Not applicable.
[e] $.7 > p > .5$ versus 27/33 (all p values from chi square tests with Yates' correction).
[f] 2,4,6-trinitrophenyl modified cells.
[g] $.7 > p > .5$ versus 27/42.
[h] $.05 > p > .02$ versus 27/42.
[i] Influenza A-virus infected cells.
[j] $.05 > p > .02$ versus 18/29.

Epa-1 CTL, Epa-1 antigen shed by the C57BL/6 EC might be presented to the CTL by syngeneic APC. The fact that ulcerative bystander reactions in the Epa-1 system are evoked with syngeneic CTL–target-cell mixtures in H-2–incompatible hosts (TABLE 3) indicates that if presentation of Epa-1 by host APC occurs, it occurs in nonrestricted fashion. Ulcerative bystander reactions, however, are usually not evoked in H-2–incompatible hosts in the TNP-hapten and influenza-virus systems (TABLE 3). Moreover, although we have evoked ulcerative bystander reactions with cloned Epa-1–specific CTL, all of the H-2–restriction tests of bystander reactivity in this antigen system were conducted with unpurified, bulk CTL populations that might have included macrophages or other APC. Thus, currently we also cannot dismiss the possibility that H-2–compatible donor APC in bystander-reaction inocula present antigen shed from the admixed allogeneic target cell to the CTL. Van Loveren *et al.*,[32] however, described a nonadherent

Lyt-1$^+$ mouse T-cell population that mediates an obligatory early component of DTH in non-MHC restricted fashion. If, as we suspect, mechanisms similar to those evoked in DTH are critical components of bystander reactions, lymphokines released during the initial CTL–target-cell interaction might activate these nonrestricted helper T cells, which in turn might release a cascade of cellular reactions[33] that eventuate in tissue destruction. Currently, we are investigating all of these possible explanations (which unfortunately are not mutually exclusive) of the apparent lack of MHC restriction of bystander reactions in some of the antigen systems we have studied. (Both cloned[4,5] and bulk-culture[30] Epa-1–specific CTL are H-2–restricted in their ability to evoke transfer reactions.)

Regardless of whether they truly defy MHC restriction, bystander reactions, as well as transfer reactions, are quite relevant to the theme of this volume, the biology of cytotoxic T cells and their role in disease. The destruction of allogeneic tissue in transfer reactions is clear evidence of the capacity of CTL to mediate allograft rejection. Thus, our demonstration that CTL, extracted and cloned from skin-cell–impregnated sponge-matrix allografts and from lymph nodes draining the sites of real skin allografts, destroy allogeneic skin[5] fulfills a form of Koch's postulates regarding CTL as mediators of allograft immunity[17] and adds to the growing evidence that CTL with the murine equivalent of the CD8 phenotype effect the acute rejection of allografts by sensitized hosts.[34,35] Our results should not be construed, however, as disputing a role in allograft rejection for other functionally distinct T lymphocytes, such as those with the CD4 (helper) phenotype that lyse target cells expressing class II MHC antigens and that mediate DTH.[17,18]

As already indicated, the tissue destruction seen in transfer reactions appears to result directly from an attack by CTL upon the allogeneic tissue into which they are inoculated: the capacity of CTL to mediate an ulcerative transfer reaction is dose-dependent, antigen-specific, and MHC-restricted.[3–5] By contrast, the destruction of "innocent cells" in bystander reactions appears to be mediated through nonspecific inflammatory cells and factors of host origin activated by the initial antigen-specific CTL–target-cell interaction. The evidence for this is twofold. First, there is no detectable nonspecific cytotoxicity by Epa-1–specific CTL *in vitro* (the only CTL we have tested so far in this regard): the addition of increasing numbers of unlabeled ("cold") Epa-1$^+$ EC target to cocultures of Epa-1–specific CTL and ^{51}Cr-labeled Epa-1$^-$ EC targets in reverse competitive ("cold-target") inhibition assays does not increase the release of ^{51}Cr above the background for the latter targets.[28] Second, supernatant fluid concentrated from cocultures of Epa-1–specific CTL and Epa-1$^+$ targets has no detectable cytotoxic effect on Epa-1$^-$ targets *in vitro* or when injected into the skin of Epa-1$^-$ hosts.[28] Although based on negative data, these findings suggest that soluble factors generated by the interaction of anti-Epa-1 CTL and their antigen-specific targets apparently are not directly responsible for the destruction of bystander cells. Thus, we feel that the bystander phenomenon more likely reflects the contribution of host regulatory and inflammatory cells not present in the artificial microenvironment of *in vitro* cell-mediated cytotoxicity assays,[27] which are activated and/or recruited at the site of inoculation of the CTL–target-cell mixture. These cells may in turn mediate nonspecific tissue destruction themselves, as would be expected of activated macrophages and natural killer cells, or indirectly, through the release of factors such as lymphotoxin, tumor necrosis factor, or toxic lysosomal enzymes. We suggest that this entire cascade of events might be triggered by lymphokine(s) released during the initial antigen-specific CTL–target-cell interaction.[32,33] Presumably, the same series of events occurs in transfer reactions, raising the inter-

esting question of the extent to which the nonspecific mechanisms activated in bystander reactions normally contribute to the tissue destruction observed in such settings as allograft rejection, tumor immunity, and viral infections.

The concept that innocent bystander cells and tissue can be damaged as a consequence of an immune response activated by the recognition of a specific antigen, although not widely appreciated, is not new. Thus, Mintz and Silvers'[36] elegant demonstration of the focal rejection of chimeric skin grafts from allophenic (tetraparental) donors composed of mixtures of syngeneic and allogeneic cells is generally regarded as definitive evidence of the specificity of allograft rejection. The evidence of nonspecific rejection in their study, however, is often overlooked: when the majority of the cells in the chimeric grafts were allogeneic, the entire grafts were rejected. In Mintz and Silvers' own words, "nonspecific necrosis can sometimes contribute to the death of the neighboring cells if enough target cells are originally implicated in the rejection."[36]

But the latter findings do not constitute proof that the syngeneic elements of the grafts were rejected in an antigenically nonspecific manner. Dvorak *et al.*[37] showed that the vasculature of split-thickness human skin allografts represents a critical target of the first-set rejection response. One might therefore postulate, as suggested by Dr. Don W. Mason (personal communication), that if the microvasculature of a chimeric graft was predominantly allogeneic, then the apparently "nonspecific" rejection seen in the Mintz and Silvers study[36] might have been caused by an antigenically specific attack on the composite grafts' vasculature. On the other hand, Dvorak *et al.*[37] also showed that the rejection of first-set human skin allografts is preceded by widespread damage of venules and arterioles in both the allograft itself and in the underlying recipient tissue, the latter certainly constituting an antigenetically nonspecific effect of the graft-rejection process. One of us observed very significantly increased contraction (presumably, a manifestation of tissue necrosis) of skin isografts taken from bone marrow chimeras, where the only targets of rejection were allogeneic "passenger leukocytes" (D. Steinmuller, unpublished observations). Similarly, Stuart *et al.*[38] observed unremitting, fatal uremia in bilaterally nephrectomized rats that received kidney isografts from allogeneic bone marrow chimeras.

Innocent bystander reactions are undisputed components of certain destructive graft-versus-host reactions in the skin,[39] kidney,[40] and small intestine[41,42] and of intense DTH reactions to tuberculin and purified proteins.[43,44] For example, Holoshitz *et al.*[44] reported that injections of T-cell lines specifically reactive with myelin basic protein or with purified-protein derivative evoked bystander encephalitis and arthritis, respectively, in mice. Niederkorn *et al.* observed that certain genetic disparities,[45] and certain tumors,[46] allogeneic or syngeneic tumors, respectively, transplanted to the anterior chamber of the mouse eye were rejected with minimal nonspecific damage to the globe; with others, acute inflammatory reactions produced massive bystander destruction of ocular tissue, resulting in blindness. The catastrophic, nonspecific form of rejection seemed to occur only when DTH was a prominent part of the immune response.[46] Bystander damage also can account for the rejection of mixtures of two syngeneic tumors, only one of which elicits a strong cell-mediated immune response.[47] In this model system, morphologic analysis indicates that rejection depends on bystander damage to the composite tumors' common vasculature.[47]

Our observation that ulcerative bystander reactions frequently occur when CTL interact with virus-infected target cells in syngeneic hosts also has clear implications for the histopathology of viral infections. For example, the widespread and frequently life-threatening results of cytomegalovirus (CMV) infection

in transplant patients are well-known.[48] Patients with the lethal CMV syndrome develop gastrointestinal hemorrhages, and CMV can be isolated from the sites of ulceration producing the hemorrhages. It is generally thought that a cytolytic effect of the virus itself causes the ulceration.[48] Our findings suggest, however, that intense inflammatory reactions triggered when the host's own CTL destroy virus-infected autochthonous cells may contribute to the tissue damage.

SUMMARY

In vitro- and *in vivo*-generated cytotoxic T lymphocytes (CTL) specific for major and minor histocompatibility antigens evoked antigen-specific full-thickness skin necrosis when injected intradermally into allogeneic mice in a variety of strain combinations. In addition, CTL–target-cell mixtures injected intradermally into hosts syngeneic to the CTL also evoked destruction of host tissue. These "innocent bystander" reactions were evoked with alloreactive CTL as well as with CTL directed against hapten (TNP)-modified and virus (influenza A)-infected target cells. Unlike the direct reactions, the bystander reactions in histocompatibility-antigen systems occurred in spite of H-2 incompatibility of the CTL, admixed target cells, and the hosts. One explanation for these results, currently under investigation, is that some bystander reactions may occur without MHC restriction. In aggregate, our findings indicate that nonspecific as well as antigen-specific reactions initiated by CTL–target-cell interactions may contribute to tissue destruction in allograft rejection, in severe forms of delayed-type hypersensitivity, and in certain viral infections.

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