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The role of accessory cells and T cell-growth factor in induction of cytotoxic T lymphocytes against herpes simplex virus antigens

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Summary. The roles of accessory cells and T cell-growth factor (TCGF) in the *in vitro* induction of herpes simplex type 1 (HSV) specific cytotoxic lymphocytes (CTL) were evaluated. Spleen cells from animals infected with HSV 4–6 weeks previously were depleted of adherent cells by passage over Sephadex G10. Unlike intact cells, such depleted spleen cells failed to respond by producing H-2 restricted virus-specific CTL upon culture for 5 days with infectious HSV. The CTL response could be restored either by adding normal genetically compatible peritoneal cells as accessory cells or by the addition of TCGF.

To obtain optimum restoration accessory cells needed to be added soon after culture initiation but with TCGF addition, partial restoration was evident when added as late as 72 hr after culture. TCGF also permitted intact spleen cells to respond to heat-inactivated virus. The results are interpreted to indicate that accessory cells are essentially required for the presentation of virus to specific helper cells with such cells responding by the production of TCGF. The results also indicate that certain forms of virus may trigger the response of CTL precursors but not the response of helper cells.

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INTRODUCTION

It is now apparent from studies in a variety of systems, that the generation of cytotoxic T lymphocyte (CTL) responses involves the essential interaction of a variety of cell types and cell products now fashionably known as interleukins (reviewed by Davidson, 1977; Wagner, Pfizenmaier & Rollinghoff, 1980, Burakoff & Mescher, 1981; Smith, Baker, Gills & Ruscetti, 1980). We have begun the analysis of the *in vitro* induction requirements for CTL generation in antigen-primed splenocytes against herpes simplex virus type 1 (HSV) and have shown that responses require the presence of accessory cells or the addition of T cell-growth factor (TCGF) to splenocytes depleted of accessory cells (Lawman, Rouse, Courtney, & Walker, 1980; Rouse & Lawman, 1980). In the present communication, we further analyse the role of accessory cells and TCGF for the induction of secondary CTL responses against HSV and show that accessory cells and TCGF are required at different stages of induction. Our data are interpreted to indicate that accessory cells are only essential for the response of helper cells to virus and that CTL precursors can respond optimally to free viral antigens providing TCGF is additionally present.

MATERIALS AND METHODS

Cells and viruses used

Strain L929 cells (H-2^k) and strain BALB/c 3T3 clone

A31 (abbreviated A31) were grown in McCoy 5A medium supplemented with 5% donor calf serum. Cell strain HEp-2, was cultured in McCoy's 5A plus 10% donor calf serum. HSV-1 strain KOS was grown in HEp-2 cells by infecting cells at low multiplicity and harvesting cell-associated virus as described previously (Lawman *et al.*, 1980).

Mouse immunization and preparation of spleen cells
Young C3H/HEJ mice (H-2^k) and BALB/c mice (H-2^d) were obtained from the University of Tennessee Medical Research Hospital or from Cumberland View Farms, Clinton, Tenn. F_1 hybrid (BALB/c × C3H/-HEJ)F_1 mice were bred in our own animal facility. Animals were infected intraperitoneally with 0·1 ml inocula containing 10^7 plaque forming units (PFU) of infectious HSV-1 KOS. At least 4 weeks following infection, mice were killed by cervical dislocation and their spleens were removed aseptically for preparation of single cell suspensions as described elsewhere (Lawman *et al.*, 1980). To measure cytotoxicity, spleen cells were adjusted to various concentrations in RPMI 1640 containing 5% FCS, penicillin (100 u./ml), and streptomycin (100 μg/ml) and buffered to pH 7·2 with Hepes (RPMI-HEPES). For *in vitro* culture, spleen cells were adjusted to 2×10^6/ml in RPMI 1640 containing 10% FCS, 2 mM glutamine, penicillin (100 u./ml), streptomycin (100 μg/ml), and 5×10^{-5} M 2-mercaptoethanol and added to plastic tissue culture plates at a density of 10^6 per cm^2 surface area. The spleen cell cultures were incubated in a humidified CO_2 (5%) incubator for 5 days, after which time cells were harvested, washed twice in medium, and adjusted to the desired concentration for cytotoxicity in RPMI-HEPES. For *in vitro* stimulation, either viable HSV-1 or heat-inactivated (60 min at 56°) virus was added directly to culture dishes containing spleen cells.

Anti-Thy 1·2 antiserum was provided by Dr Jon Sprent (Wistar Institute, Philadelphia) and rabbit low tox complement from Cedarline Laboratories (Hicksville, N.Y.). Cells were incubated for 45 min at room temperature with a 1:256 dilution of antiserum, followed by 30 min at 37° with complement.

Preparation of T cell-growth factor (TCGF)-containing supernatant fluid
Spleen cells were isolated from Lewis rats and suspended at a concentration of $1{\cdot}25 \times 10^6$/ml in RPMI 1640, 10% FCS. Aliquots (20 ml) of this suspension were placed in plastic 100 mm petri dishes and stimulated with 3·0 μg/ml of concanavalin A (Con A, Pharmacia, Uppsala, Sweden). Cells were cultured for 24 hr at 37° in a humidified CO_2 incubator, after which the supernatant fluids were harvested and freed of cells and large cellular debris by centrifugation at 2000 ***g*** for 30 min. Con A was neutralized by adding 0·1 mg/ml of α-methyl mannoside, or removed by absorption to Sephadex G-25 (Glasebrook & Fitch, 1980). Supernatant fluids were then sterilized by filtration through a 0·2 μ Nalgene membrane filter, and stored in aliquots at −70°.

Isolation of resident peritoneal cells (PC)
Mice were killed by cervical dislocation and 10 ml of chilled phosphate-buffered saline (PBS) were injected into the peritoneal cavity. Cells were removed and pelleted by centrifugation at 400 ***g*** for 4 min then suspended in culture media and added to cultures of splenocytes in various numbers. In some experiments, PC were incubated for 1 hr at 22° with infectious HSV-1 at a multiplicity of infection (MOI) of ten, washed twice with HBSS and then added to cultures in graded numbers.

Removal of adherent cells on Sephadex G-10
This was done as described by Ting & Rodrigues (1980). Briefly, sterile Sephadex G-10 was equilibrated with RPMI 1640, 10% FCS and packed to a volume of 20 ml in syringes which had been plugged with a small piece of glass wool. The columns were adjusted to 37°. Intact spleen cells were thoroughly mixed into the G10 by pipetting and incubated for 1 hr at 37°, with occasional remixing. Non-adherent cells were eluted with 20–25 ml of warm medium and pelleted by centrifugation at 400 ***g*** for 8 min. These cells were resuspended and subjected to a second passage over Sephadex G-10.

Cytotoxicity assays
The details of these assays were published previously (Lawman *et al.*, 1980). Briefly L or A31 cells suspended in McCoy 5A medium were infected with HSV-1 at a MOI of 10 for 60 min at 37° then washed and 5×10^6 cells in 1·0 ml labelled for 1 hr with 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.). After labelling, cells were washed three times with medium, suspended at 10^5/ml in RPMI-HEPES and 100 μl volumes added to effector cells in U-shaped microtitre plates. Cytotoxicity assays were begun 5 hr after the initiation of infection with HSV-1 and were carried out for 5 hr in a 5% CO_2 humidified incubator. Standard errors were low (<4%), and results are

reported as mean percentage specific release values computed according to the following formula: percentage specific ^{51}Cr release = [(effector cell release − medium control release)/(total releasable ^{51}Cr-medium control)]. The total releasable ^{51}Cr was obtained by exposing target cells to 1% triton X100.

RESULTS

Requirement for syngeneic accessory cells during induction of anti-HSV CTL

As reported before (Rouse & Lawman, 1980), when intact spleen cells from mice infected 4–6 weeks previously with HSV-1 were cultured *in vitro* with infectious virus, specific cytotoxic activity was generated which was presumably T-cell mediated since the activity was H-2 restricted and was destroyed by treatment with anti-Thy 1 plus complement (Table 1). However, CTL induction did not occur upon removal of adherent cells from the primed splenocytes, by passage over Sephadex G-10. These depleted cell populations could be restored to full activity by the addition of syngeneic, but not allogeneic, peritoneal cells to a concentration of 2·5% of the total cell population (an accessory cell number found to be optimum). To establish at which stage in the induction process the accessory cells were required, experiments were performed in which G10-depleted primed splenocytes were placed in culture, virus added, after which optimal numbers of syngeneic PC were added at different intervals. Cultures were maintained for 5 days and the levels of cytotoxicity measured. As can be seen, the addition of PC at culture initiation or at 24 hr, permitted full restoration of activity but when the addition was delayed to 48 hr or later, little or no cytotoxicity was generated (Table 1). However, it is also apparent from Table 1 that if measures were taken to remove non-cell associated virus from depleted cells before the addition of PC at 24 hr, then the cytotoxic response was markedly reduced.

Not shown in Table 1 was the fact that if extra infectious virus was added along with the PC at 48 hr and 72 hr and the cultures subsequently permitted to

Table 1. Accessory cell requirement for induction of cytotoxic T lymphocyte responses in HSV primed splenocytes*

Responder population	Accessory cells	Time added (hr)	Viral antigen	Percentage specific lysis†		
				L-HSV (H-2^k)	A31-HSV (H-2^d)	L(H-2^k)
Undepleted	—	—	+	81·7	17·3	5·1
Undepleted	—	—	−	8·0	0·7	1·7
Undepleted	—	—	+	7·2	1·2	1·9
Depleted‡	—	—	+	3·1	1·3	0·8
Depleted	H-2^d	0	+	16·4	2·5	2·0
Depleted	H-2k	0	+	82·4	12·9	4·8
Depleted	H-2^k	24	+	78·0	ND	8·1
Depleted	H-2^k	48	+	15·3	ND	4·2
Depleted	H-2^k	72	+	3·2	ND	1·2
Depleted	H-2^k	96	+	1·8	ND	0·2
Depleted	H-2^k	24	+§	32·1	ND	3·8

* Spleen cells from C3H/HEJ (H-2^k) mice were cultured for 5 days intact or depleted of adherent cells by two consecutive passages over Sephadex G10 at a cell density of 10^7 cells in 5 ml culture fluid. Peritoneal cells from C3H or BALB/c (H-2^d) were added at different time intervals at cell concentrations of $2{\cdot}5 \times 10^5$ per culture—a concentration found previously to be optimum (Rouse & Lawman, 1980).

† The values shown represent means of quadruplicate cultures run at effector to target cell ratios of 25:1. The standard errors for the 6 hr assays were always below 4%. The H-2^k targets were L929 cells and the H-2^d targets clone A31. Three repeat experiments gave a similar pattern of results.

‡ Effector cells treated with anti-Thy 1·2 serum plus complement before measurement of cytotoxicity.

§ In this group, the depleted cells were washed three times to remove non-cell associated virus before the addition of PC at 24 hr.

proceed for a further 5 days, virtually full cytotoxic activity occurred. Thus, the depleted cells were still viable and responsive to induction after 2 days or more in culture if adequately stimulated by virus when presented with an accessory cell.

Our results thus far indicate the requirement for an adherent accessory cell, presumably a macrophage, and that this accessory cell is required early during induction and must be syngeneic with the responding splenocytes to perform the accessory function. To investigate further the requirement for genetic restriction during induction of anti-HSV CTL responses, experiments were performed in which HSV-1 primed (BALB/c × C3H)F_1 splenocytes were depleted of adherent cells and their responses to virus presented in the context of parental PC determined. In these experiments, the PC were reacted with virus at 37° for 60 min and washed twice before addition to depleted cultures. After 5 days of culture, the cytotoxic activity of surviving cells was measured against infected target cells of both parental genotypes. The results, expressed in Table 2, clearly indicate a preferential restoration of CTL activity against the infected target sharing H-2 identity with the accessory cell used for restoration. Thus, the accessory cell–T cell interaction showed H-2 restriction with presumably only those virus-specific T cells in an F_1 population committed to one of the two haplotypes being activated when presented with virus. In some instances, when large numbers of PC were added, some suppression of the maximum response was observed. Such suppression has been observed by others (Lause, 1979; Ting & Rodrigues, 1980) and may reflect a normal regulatory function of accessory cells. The results of these experiments indicate that accessory cells were playing an antigen presentation role rather than simply providing a feeder effect, since if the latter was occurring then parental type PC should restore the response of F_1 cells against both target haplotypes.

Role of TCGF in induction of anti-HSV cytotoxic T lymphocytes

It is apparent from the results in several systems (Wagner *et al.*, 1980) including induction of anti-HSV CTL (Rouse & Lawman, 1980) that the requirement for accessory cells during induction may be replaced by certain soluble cell products. In Table 3, the results are presented of an experiment designed to investigate the requirement for TCGF for induction of anti-HSV CTL. In the experiment, G-10-depleted primed splenocytes were reacted with virus, TCGF added at

Table 2. Reconstitution of depleted HSV-primed (BALB/c × C3H)F_1 splenocytes with parental type accessory cells

Responder population	Accessory no.	Cell strain	Viral antigen	Percentage specific lysis†	
				L-HSV (H-2^k)	A31-HSV (H-2^d)
Undepleted	—		+	81·2	76·2
,,	—		—	2·7	1·5
Depleted*	—		+	1·8	3·4
,,	10^5	H-2^d	+	2·8	7·1
,,	$2·5 \times 10^5$	,,	+	7·8	25·6
,,	5×10^5	,,	+	14·5	55·2
,,	10^6	,,	+	12·6	47·5
,,	10^5	H-2^k	+	9·7	3·4
,,	$2·5 \times 10^5$	,,	+	36·0	14·1
,,	5×10^5	,,	+	74·7	29·1
,,	10^6	,,	+	61·2	17·3
,,	5×10^5	F_1	+	68·5	60·1

* Cells from F_1 mice were depleted as described in the footnotes to Table 1. The accessory cells were peritoneal cells for C3H (H-2^k), BALB/c (H-2^d) or F_1 mice. These cells were reacted with HSV-1 at a multiplicity of infection of 10 for 60 min at 37°, washed twice then added to cultures.

† The chromium release assay was performed for 6 hr at an effector to target cell ratio of 25:1. Standard errors ranged between 1%–4%. Two repeat experiments gave essentially similar results.

Table 3. Induction of cytotoxic T-cell responses in adherent cell-depleted HSV-primed splenocytes by addition of T cell-growth factor*

Responder population	Viral antigen	TCGF and time added (hr)	Percentage specific lysis		
			L-HSV (H-2^k)	A31-HSV (H-2^d)	L(H-2^k)
Undepleted	+	–	83·5	13·5	4·9
Undepleted	–	—	9·8	ND	2·1
Depleted	+	—	3·7	1·5	1·0
Depleted	+	0	88·3	14·2	8·6
Depleted	+	24	85·6	ND	9·7
Depleted	+	48	63·0	ND	7·9
Depleted	+	72	23·6	ND	2·3
Depleted	+	96	3·2	ND	0·8
Depleted	+†	24	78·3	ND	5·6
Depleted	+†	48	59·1	ND	3·2

* Experimental design is described in Table 1 except that TCGF was added to a final concentration of 50% instead of the addition of accessory cells. The TCGF was prepared by stimulating rat splenocytes with Con A for 48 hr. Experiment repeated four times with similar results.

† In these groups the virus-depleted cells were washed three times to remove non-cell-associated virus before the addition of TCGF.

varying intervals and cultures maintained for 5 days. Such experiments were run concurrently with the types of experiments recorded in Table 1. It is apparent that the addition of TCGF permitted the induction of substantial CTL responses when added either at the time of culture initiation or as late as 48 hr afterwards. Even when added at 72 hr, a partial CTL response occurred. Furthermore, if the virus-stimulated depleted cells were washed to remove any non-cell associated virus before the addition of TCGF at 24 and 48 hr, then the response of these cultures was comparable with unwashed cultures. Thus, TCGF can be added later than accessory cells and still permit the generation of a CTL response indicating that TCGF may act later in the induction process then do accessory cells.

TCGF is known to act non-specifically in other systems studied (Smith, 1980; Wagner *et al.*, 1980) and it was therefore no surprise to find that upon addition of TCGF to F_1-depleted splenocytes, CTL responses active against both parental haplotypes were generated. This proved to be the case as indicated in Table 4,

Table 4. Restoration of cytotoxic response of adherent cell-depleted (BALB/c × C3H)F_1 HSV-primed splenocytes with cell growth factor*

Responder population	Viral antigen	TCGF and time added (hr)	Percentage specific lysis	
			L-HSV (H-2^k)	A31-HSV (H-2^d)
Undepleted	+	Not added	74·4	42·6
Undepleted	–	Not added	13·6	8·2
Depleted	+	Not added	6·4	3·0
Depleted	+	0	66·4	36·3
Depleted	+	24	67·5	33·0
Depleted	+	48	58·3	21·6
Depleted	+	72	14·7	9·7
Depleted	+	96	6·9	3·6

* Experimental design as described in footnotes to Tables 2 and 3. Experiment repeated three times with similar results.

Table 5. Failure of induction of cytotoxic responses in HSV-primed splenocytes by stimulation with heat-inactivated virus

Responder population	Viral antigen	TCGF addition	Percentage specific lysis	
			L-HSV	L
Undepleted	Infectious*	None	83·3	5·9
Undepleted	HI	None	12·1	2·6
Undepleted	None	+	13·4	12·4
Undepleted	HI	+	69·5	9·8
Undepleted	None	None	10·2	3·4
Depleted	Infectious	None	14·8	4·1
Depleted	HI	None	4·7	6·1
Depleted	HI	+	50·2	3·6
Depleted	Infectious	+	63·6	4·2

* Virus (10^7 PFU) added to cultures of 10^7 cells. The same viral stock was heated at 56° for 60 min to produce the heat-inactivated virus.

with the responses to both H-2^d and H-2^k virus-infected targets being substantially restored by TCGF. It should also be noted that the addition of TCGF to depleted F_1 splenocytes at different times showed a pattern similar to that observed in the responses of C3H-depleted splenocytes to TCGF.

Response of intact and depleted primed splenocytes to heat-inactivated virus

We have observed that whereas infectious or u.v. inactivated HSV will induce a CTL response in intact primed splenocytes, heat-inactivated virus fails to induce a response unless TCGF is added to such HI stimulated cultures (Rouse & Lawman, 1980). As shown in Table 5, the addition of TCGF to virus-stimulated adherent cell-depleted primed splenocytes permitted the generation of a substantial CTL response to HI virus indicating that CTL precursors can respond to such virus in the absence of accessory cells. The addition of TCGF at different times to HI-stimulated depleted primed splenocytes showed similar results to those recorded in Table 2 (data not included). Whereas the addition of TCGF to intact or depleted cultures permitted the response to HI virus, the addition of additional accessory cells failed to restore a response (data not included).

DISCUSSION

The intent of the present communication was to investigate and compare the requirements for accessory cells and TCGF in secondary *in vitro* CTL responses of spleen cells to HSV antigens. We demonstrated that CTL induction did not occur if adherent cells were removed from HSV-primed spleen cells before antigen stimulation. Thus secondary CTL responses to herpes antigens require the participation of accessory cells such has also been shown for the induction of CTL to subcellular (Degiovanni, Cerottini & Brunner, 1980; Weinberger, Hermann, Mescher, Benacerraf & Burakoff, 1980) and to tumour antigens (Fyfe & Finke, 1979; Glaser, 1980; Swierkosz, Rock, Marrack & Kappler, 1977; Woodward, Fernandez & Daynes, 1979). Although only few studies deal with CTL induction against viral antigens, it appears that accessory cells may (Pang, McKenzie & Blanden, 1976; Hapel, Bablanian, & Cole, 1978; Rouse & Lawman, 1980) or may not be involved (Koszinowski & Gething, 1980). Apparently with viral antigens able to fuse into cell membranes as for example Sendai virus infection, accessory cells may not be necessary (Koszinowski & Gething, 1980). In the system we investigated, two ways were found of restoring the induction of anti-herpes CTL in adherent cell-depleted splenocytes. These were either to add back normal peritoneal cells or to add TCGF. In both cases, full restoration could be achieved under optimum conditions. For accessory cells, this was between 2·5% and 5% of the total cell population. In the present communication, the identity of the accessory cell responsible for the restoration was not examined but if parallels can be drawn with other systems examined (Davidson, 1977; Wagner *et al.*, 1980; Woodward *et al.*, 1979), we assume that the macrophages in the PC

population were principally involved. However, with respect to the induction of CTL responses to herpes antigens, we have shown that several cell types other than macrophages can discharge accessory cell function, these including Ia negative fibroblasts (Rouse & Lawman, 1980) as well as Ia positive, B cells, null cells and dendritic cells (Schmid unpublished observations).

Our experiments were directed at comparing the restorative effects of accessory cells and TCGF. Clear differences were noted in the time at which it was necessary to add these components in order to achieve optimum CTL induction. For instance, to obtain optimum induction by restoration with accessory cells, the cells needed to be added soon after culture initiation and viral stimulation. It was possible to delay the addition for 24 hr, and still obtain optimal induction provided free virus was added to stimulate cultures initially. However, when efforts were made to remove unbound virus from depleted cells at 24 hr before adding PC, only a suboptimum CTL response was obtained. These experiments indicate that one phase of the anti-herpes induction process requires presentation of virus by way of an accessory cell. Furthermore this viral presentation had to be made by a genetically compatible accessory cell. Induction did not occur upon restoration with allogeneic PC and after the addition of PC of one parental haplotype to F_1-depleted cells, CTL were induced that were cytotoxic only to target cells of the same haplotype as used in restoration. Thus as shown initially by Rosenthal's group (Rosenthal & Shevach, 1973) and subsequently confirmed by others in different systems (Farr, Wechter, Kiely & Unanue, 1979; Schwartz, Yano & Paul, 1978; Swierkosz *et al.*, 1977), there exists in F_1 animals two clones of antigen reactive T-cell subsets each reactive with antigen in the context of only one of the two MHC haplotypes.

Our observations that induction could be achieved by the addition of TCGF to virus-stimulated cultures implies that accessory cells are not essential for all phases of the CTL response. Indeed, in contrast to restoration with accessory cells, TCGF could be added as late as 48 hr after culture initiation and still induce optimal CTL responses. Furthermore, virus-treated depleted cells washed to remove unbound virus, showed a similar pattern of response to TCGF addition unlike that observed with accessory cell supplementation (*vide supra*). Attesting to the non-specific activity of TCGF function was the observation that the addition of TCGF to F_1 depleted splenocytes permitted the generation of CTL able to kill virus-infected targets of both haplotypes. We interpret the results of our present experiments to imply that the H-2 restricted accessory cell requirement for anti-herpes CTL induction is principally at the level of the antigen-specific cell that generates TCGF rather than the CTL precursors. Others have, in fact, indicated that TCGF is produced by helper cells (Okada, Klimpel, Kuppers & Henney, 1979; Shaw, Caplan, Paekau, Pilarski, Delovitch & McKenzie, 1980; Wagner & Rollinghoff, 1978) which are T cell subsets distinct from CTL precursors and distinguishable by their expression of Ly alloantigens (Cantor & Boyse, 1975; Wagner & Rollinghoff, 1978). Furthermore, for helper cells to produce TCGF, they require stimulation by mitogen and presumably antigens, in the context of a genetically compatible macrophage (Swain & Dutton, 1980). It seems likely that the requirement for H-2 restriction and accessory cells in anti-herpes CTL induction is principally at the level of helper cells and these function to produce TCGF which in turn trigger CTL precursors that have bound antigen in the absence of macrophages to differentiate into CTL effectors. Whereas the entire CTL induction takes 5 days, TCGF can be provided at least as late as 48 hrs and still be fully effective. Presumably during the first 48 hr of culture TCGF is being generated by herpesvirus-specific helper cells.

The hypothesis that CTL precursors have different antigen presentation requirements than helper cells may also be the explanation for our observation that HI virus failed to induce CTL. However, induction occurred upon the addition of TCGF but not by adding extra accessory cells. We presume that heated virus, perhaps because it fails adequately to engage macrophages, cannot trigger helper T cells. Experiments to verify this hypothesis are in progress.

The observation of H-2 restricted restoration of F_1-depleted cells with parental PC, might be taken as an argument that the CTL precursor as well as the helper cells require to receive antigen in the context of a genetically compatible accessory cell. Thus, if helper cells react with antigen and produce TCGF, since this acts non-specifically, both clones of antigen-specific T cells in the F_1 population should be triggered to become cytotoxic. However, in these experiments the only source of antigen was in the membrane of the PC and hence it is possible that H-2 restriction was observed because only these cells make effective interaction with those clones in the F_1 T-cell population reactive with the compatible haplotype. Indeed

we have observed that if extra, i.e. non-cell bound antigen, was added to these cultures, some breakdown in restriction occurred implying that when virus is available other than in an accessory cell membrane other clones of CTL precursors may become triggered.

An alternative explanation could be that although only the helper cells show H-2 restriction for their stimulation the amount of IL-2 produced in cultures in response to antigen may be so limited that it is bound by closely adjacent cells which may be only those CTL of the same haplotype. We are currently pursuing experimental approaches designed to substantiate our notion that CTL precursors, but not helper cells, specific to herpes virus antigens can respond to antigen in a non H-2 restricted mode as well as to different types of antigen preparations. We are also investigating the putative role of anti-herpes CTL in host defence *in vivo*.

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