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Hamster T Cells Participate in MHC Alloimmune Reactions but Do not Effect Virus-Induced Cytotoxic Activity

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Abstract. The participation of hamster T cells in a variety of putative MHC-determined reactions was studied utilizing a well-characterized, highly selective goat anti-hamster thymocyte (GαHT) serum. Hamster lymphoid cell suspensions treated with GαHT lose much of their capacity to induce local graft-versus-host reactions and to function as responder cells in mixed lymphocyte reactions. In contrast to the participation of hamster T cells in alloimmune reactions (MLR and GVHR), virus-induced, cytotoxic activity in hamsters undergoing acute virus infection is not T-cell-mediated. This latter finding was rather surprising in view of the major role played by cytotoxic T effector cells in comparably infected mice and rats. These results suggest that, although hamsters are able to respond to putative class II MHC disparities in allogeneic reactions, MHC-encoded molecules, presumably class I, are not utilized for induction of effective cytotoxic activity in response to acute virus infection in this species. The implications of these findings are discussed in relation to our present understanding of the hamster MHC.

Introduction

During the past few years, this laboratory has been concerned with the description and characterization of the major histocompatibility complex (MHC) of Syrian hamsters. Studies in a variety of other species have clearly indicated an association between T-cell-mediated immune reactions and the MHC. Participation of T cells in MHC-determined alloimmune responses such as mixed lymphocyte reactions (MLR) (Mosier and Cantor 1974), cell-mediated lympholysis (CML) (Cerottini and Brunner 1974) and graft-versus-host reactions (GVHR) (Cerottini et al. 1971) has been extensively documented (Albert and Götze 1977, Geczy and de Weck 1977, Gunther and Stark 1977, David 1977). In addition to their role in allogeneic responses, T cells are also thought to play an important physiological role in the host response to viral infection (Doherty et al. 1976).

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Immune reactions mediated by T cells in hamsters have not been fully described. In the few studies in which functional hamster T cells were investigated, they were shown to be involved in acute skin-graft rejection (Roosa et al. 1965) and resistance to certain tumors (Blasecki 1977). To date, no studies have been described which investigated the role of hamster T cells in MHC-determined responses.

Hamster MLR, GVHR, and CML have been previously described with special reference to the allogenic disparities responsible for elicitation of such responses (Duncan and Streilein 1977, 1978 a, 1979 b). Although not formally proven at the time, it was assumed that these putative MHC-directed responses were mediated by cells of thymic origin. We have recently described cell-mediated, cytotoxic activity in lymph nodes and spleens of hamsters undergoing acute virus infection (Nelles and Streilein 1980). Surprisingly, no evidence of genetic restriction of cell-mediated killing was observed in this system despite the use of several different hamster strains putatively disparate at the hamster MHC, *Hm-1*.

The study presented herein is a direct attempt to determine the participation of hamster T cells in a variety of putative MHC-determined responses in the hope of gaining a greater understanding of the hamster MHC. This is particularly important in view of the results obtained in the virus-induced cytotoxic effector system in which no genetic restriction was observed. Although we show that hamster T cells are involved in responses resulting from allogeneic disparity (MLR and GVHR), we are unable to find evidence of a cytotoxic effector T cell in the virus-induced cytotoxic system.

Materials and Methods

Experimental animals. Syrian hamsters were purchased from the Charles River Lakeview Company, Newfield, New Jersey (MHA and (MHA × CB)F_1) or were bred in our own colony (LSH and (LSH × CB)F_1). C3H strain mice were bred in our own colony. Animals of both sexes were utilized at 3 to 9 months of age.

Cellular suspensions. Cell suspensions were prepared as previously described (Duncan and Streilein 1978 b). Briefly, thymus, pooled lymph nodes, and spleens were gently minced over fine mesh screens. Bone-marrow suspensions were obtained from femurs by flushing with cold media. Single cell suspensions were collected in balanced salt solution (BSS) containing either 2% fetal calf serum (FCS) or 5 mg/ml bovine serum albumin (BSA) depending on the experiment. After washing in BSS, cells were resuspended to appropriate concentrations,

Complement mediated cytotoxicity. Twofold dilutions of antiserum or normal serum were made in BSS containing 5 mg/ml BSA in round bottom 96 well plates (Cooke Laboratory Products, Alexandria, Virginia) in a volume of 0.05 ml. Single cell suspensions of thymus, lymph node, ACT-treated spleen (Boyle 1968), and bone marrow, at a concentration of 20×10^6/ml, were mixed with various dilutions of antiserum or normal serum in a total volume of 0.1 ml. After incubation for 20 min at 37° C, cells were pelleted and resuspended in 0.1 ml of guinea pig serum diluted 1 : 8. Cells were incubated further for 30 min at 37° C at which time the percentage of cells killed was determined by trypan blue uptake.

Popliteal lymph-node hypertrophy assay (PLN). Local graft-versus-host reactions (GVHR) were studied using the PLN assay as previously described (Duncan and Streilein 1978 a). In short, 1 week after inoculation of 10×10^6 normal LSH strain lymph-node cells (experimental) and normal (LSH × CB)F_1 lymph-node cells (control) into contralateral rear footpads of (LSH × CB)F_1 animals, the draining popliteal lymph nodes were removed and weighed. The PLN index was calculated as follows:

$$\frac{\text{weight of experimental node/body weight}}{\text{weight of control node/body weight}} \times 100$$

Mixed lymphocyte reaction (MLR). Mixed lymphocyte reactions were performed essentially as described (Duncan and Streilein 1978 a) utilizing 1×10^6 responder lymph-node cells and 1×10^6 irradiated (3000 R) stimulator spleen cells in 0.2 ml RPMI-1640 (Grand Island Biological Co. Grand Island, New York,) without serum. Cultures were harvested 3 days later after a 24 h pulse of 0.1 μCi of ^{3}H-thymidine (Schwarz/Mann, Orangeburg, New York). Stimulation indices were calculated according to the following formula:

$$\frac{\text{cpm experimental (responder + irradiated stimulator)}}{\text{cpm control (responder + irradiated responder)}}$$

Mitogen induced proliferation. Concanavalin A(Con A) (Sigma Chemical Co., St. Louis, Missouri) and lipopolysaccharide (LPS) (Difco, Detroit, Michigan) induced proliferation assays were performed as described elsewhere (Streilein and Hart 1976). Lymph-node cells (1×10^6) were cultured in RPMI-1640 under serum-free conditions at 37° C in a volume of 0.2 ml. Con A (0.2 μg/well) and LPS (20 μg/well) were added at the initiation of cultures in a volume of 0.025 ml. Cultures were harvested at 3 days after a 24 h pulse of 0.1 μCi of ^{3}H-thymidine. Stimulation indices were calculated according to the following formula:

$$\frac{\text{cpm experimental (mitogen)}}{\text{cpm control (media)}}$$

Primary antibody-forming cell (AFC) assay. Hamsters were injected intravenously and in the footpads with 1×10^9 and 2×10^9 sheep red blood cells (SRBC), respectively. Four days later, draining lymph nodes and spleen were removed, made into single cell suspensions, and the number of direct (IgM) antibody-forming cells (AFC) was determined by a slide modification of the Jerne plaque assay (Jerne et al. 1963) as described by Stein-Streilein and co-workers (1979).

Virus-induced cytotoxic effector cells. Hamster (MHA) and mouse (C3H) virus-induced cytotoxic effector cells were generated in vivo as described (Nelles and Streilein 1980, Zinkernagel et al. 1978 a). Briefly, hamsters and mice were inoculated intraperitoneally with approximately 8×10^6 and 3×10^6 PFU of vaccinia virus, respectively. Six days later, animals were sacrificed, spleens were removed and made into single cell suspensions, and assayed for cytotoxic activity in vitro in a ^{51}Cr release assay. Syngeneic tumor target cells (MHA strain SV40 transformed fibroblasts-MHST, L strain mouse fibroblast-L cells) were plated the night before at a concentration of 5×10^4 cells per well in 96 flat bottom trays (Falcon, Oxnard, California). The next day, target cells were labelled with 3 μCi ^{51}Cr as sodium chromate (Amersham Corp. Arlington Heights, Illinois) and infected with vaccinia virus at a multiplicity of infection of 10. Virus immune effector spleen cells in RPMI-1640 with 10% FCS were overlayed at various effector to target (E : T) ratios and incubated at 37° C for 16 h (hamster system) or 6 h (mouse system). At the termination of the assay, 0.1 ml of supernatant was removed and assayed for radioactivity as a measure of cell death. Percent specific cytotoxicity was calculated according to the following formula:

$$\frac{\text{cpm experimental} - \text{cpm media control}}{\text{cpm detergent lysis}} \times 100$$

Preparation and testing of goat anti-hamster Thymocyte Serum (GαHT). Goat anti-hamster thymocyte serum (GαHT) was produced by injection of 1×10^9 MHA strain hamster thymocytes in complete Freunds adjuvant into an adult female goat. The animal was boosted 6 weeks later with 5×10^8 thymocytes inoculated intravenously without adjuvant.

The resultant antiserum obtained was heat-inactivated at 56° C for 30 min and stored at −70° C. All absorptions were carried out at 4° C for 30 min. Five ml aliquots were absorbed twice with equal volumes of washed, packed hamster erythrocates, once with an equal volume of washed, packed sheep erythrocates, and once with 5×10^7 washed, hamster bone-marrow cells. The antiserum was further absorbed three times with a total of 1.5×10^9 viable GD-36 cells, a hamster B-cell lymphoma (Coe 1976)

which was sufficient to deplete the antiserum of cytotoxic activity towards GD-36 lymphoma cells without appreciable loss of activity to hamster thymocytes. Normal goat serum (NGS) was absorbed in parallel.

For functional testing of cells treated with the goat anti-hamster thymocyte reagent (GαHT), 0.1 ml of a 1 : 10 dilution was mixed with each 15×10^6 lymphoid cells. After incubation at 37° C for 20 min, cells were washed, resuspended in 0.1 ml of a 1 : 8 dilution of guinea pig serum and further incubated for 30 min at 37° C. Remaining cells were washed, counted, and resuspended to the appropriate concentration for use in various T-cell functional assays. Treatment with NGS and complement was always performed in parallel and serves as a control.

Results

Tissue distribution of hamster thymus-derived cells

Goat anti-hamster thymocyte serum (GαHT) specifically recognizes thymus-derived cells as shown in Figure 1. Treatment of hamster lymphoid cells with GαHT, in the presence of complement, kills 94 percent of thymocytes, 88 percent of lymph-node cells, 80 percent of spleen cells, and 12 percent of bone-marrow cells at a dilution of 1 : 5. Background levels of killing with normal goat serum (NGS) as control were approximately 25 percent for thymocytes, lymph-node cells, and spleen cells, and 7 percent for bone-marrow cells. A murine monoclonal anti-Thy 1 alloantiserum which cross-reacts with hamster lymphoid cells identifies comparable numbers of T cells in the various hamster lymphoid organs (data not shown). The relatively high proportion of T cells identified in hamster spleens, as compared to rats and mice, has been previously observed (Rheims et al. 1975).

In addition, when murine lymphoid cells are treated with the goat-anti-hamster thymocyte serum in the presence of complement, the numbers of T cells identified in thymus, lymph nodes and spleen are comparable to those obtained utilizing a rabbit anti-mouse brain antiserum (M. Nelles, unpublished observations). Taken together, these observations strongly support the idea that the goat anti-hamster thymocyte serum identifies the hamster homologue of murine Thy 1. The antiserum's pattern of cross-reactivity on various murine lymphoid populations provides evidence against

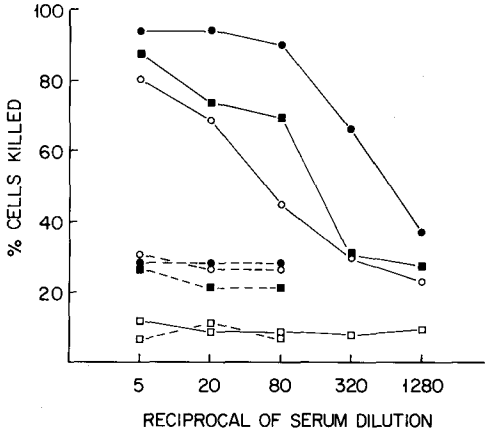

Fig. 1. Cytotoxic reactivity of GαHT (——) and NGS (– – –) against MHA strain hamster thymus (●), lymph node (■), spleen (○), and bone marrow (□) cells.

the unlikely possibility that the reagent is directed at putative hamster homologues of the Ly series of murine antigens.

Functional responses of hamster T cells tested in vitro and in vivo

In order to characterize further the GαHT reagent, hamster lymphoid cell suspensions were treated with this anti-T-cell antiserum (in the presence of complement) and the cells remaining were tested in a variety of functional assays.

A) Mitogenic responses of hamster lymphoid cells. Lymph-node cell suspensions were treated with either GαHT or NGS in the presence of complement. Remaining cells were then assayed in vitro for their capacity to undergo proliferation in response to the T-cell mitogen, Concanavalin A(Con A) and the B-cell mitogen, lipopolysaccharide (LPS). As shown in Table 1, 61 percent of the Con A-induced proliferative response of hamster lymph-node cells is reduced by GαHT treatment, while the LPS-induced proliferative response of GαHT-treated cells remains essentially unchanged (7 percent decrease). Inhibition of Con A-induced proliferation in hamsters with an anti-hamster T-cell antiserum has been described previously (Blasecki and Houston 1978). Although the level of endogenous thymidine incorporation of GαHT-treated cells was significantly above that for NGS-treated cells (2173 vs. 892 cpm, respectively), the stimulation indices of LPS-induced proliferation were equivalent (5.1 ans 5.5, respectively). It is concluded that the GαHT reagent recognized those T cells which are required for a Con A-induced proliferative response in vitro. Further, the GαHT reagent lacks reactivity for B cells as judged by the ability of cells remaining after GαHT treatment to proliferate optimally in response to the B-cell mitogen, LPS.

B) Hamster antibody-forming cells (AFC) are not T cells. In the next set of experiments, spleen and lymph nodes from hamsters injected 4 days previously with sheep erythrocytes (SRBC) were assayed by the Jerne plaque technique for the number of direct (IgM) antibody-forming cells in vitro. As is shown in Table 2, lymph-node and spleen cell suspensions treated with GαHT possess essentially

Table 1. Effect of goat anti-hamster thymocyte serum on hamster mitogenic responses to con A and LPS

Treatment*	Mitogen	CPM incorporated	Stimulation index	Percent reduction†
NGS	–	829 ± 68	–	–
	LPS	4570 ± 34	5.5	–
	Con A	17439 ± 323	21.0	–
GαHT	–	2175 ± 203	–	–
	LPS	11186 ± 1486	5.1	7
	Con A	17883 ± 1060	8.2	61

* MHA strain lymph-node cells were treated with antiserum or normal serum in the presence of complement, counted, resuspended to equivalent concentrations, and cultured under serum-free conditions for 3 days with or without mitogens. Values of cpm incorporated represent the mean ± standard error of the mean of three replicate cultures.

† As compared to stimulation indices of NGS treated lymph-node cells.

Table 2. Effect of goat anti-hamster thymocyte serum on primary anti-SRBC antibody-forming response

Cell type	Treatment*	AFC/10^6 cells†	Percent reduction‡
LN	NGS	684± 13	–
	GαHT	696± 14	0
SPL	NGS	9398±410	–
	GαHT	10747± 84	0

* LSH strain hamsters were inoculated intraperitoneally and in the footpads with SRBC. Four days later, spleen and draining lymph nodes were obtained, made into single cell suspension, and treated with either NGS or GαHT in the presence of complement.
† Based on cell counts prior to treatment with NGS and GαHT.
‡ As compared to lymphoid cells treated with NGS.

identical numbers of AFC as compared to cell suspensions treated with NGS (lymph node 696 vs. 684/10^6; spleen: 10 747 vs. 9393/10^6, respectively) Therefore, the GαHT reagent used in these and subsequent experiments selectively recognizes hamster T cells as judged by tissue distribution criteria and functional (mitogenic) studies. The antiserum lacks reactivity for a hamster B-cell lymphoma (*Materials and Methods*), bone-marrow cells (Fig. 1), LPS-reactive cells (Table 1), and cells secreting specific antibody (Table 2).

C) Alloimmune reactions mediated by hamster T cells. It has been previously shown that parental strain lymphoid cells injected into footpads of adult F_1 hybrid hamsters induce significant popliteal lymph-node hypertrophy (Duncan and Streilein 1978 a). The dependence of this alloimmune response on hamster T cells was examined by treating parental lymphoid cell suspensions with GαHT prior to inoculation into F_1 hybrid recipient foot pads. As shown in Table 3, LSH strain lymph-node cells treated with GαHT lose much of their capacity to elicit local GVHR when injected into (LSH × CB)F_1 hybrid animals. The resultant lymph-node hypertrophy was reduced by 65 percent as compared to that induced by parental lymphoid cells treated with NGS or media alone (PLN indices of 3.9, 11. 9, and 11.1, respectively).

Table 3. Effect of goat anti-hamster thymocyte serum on the ability of hamster lymph-node cells to elicit graft-versus-host reactions

Treatment*	PLN index	Percent reduction†
–	11.1±0.6	–
NGS	11.9±5.0	–
GαHT	3.9±1.0	65

* LSH strain hamster lymph-node cells were untreated, NGS+C′ treated, or GαHT+C′ treated and injected into one rear footpad of an (LSH × CB)F_1 animal. Untreated (LSH × CB)F_1 lymph-node cells were injected into the contralateral footpad and the degree of popliteal lymph-node hypertrophy was determined 7 days later. Two animals were utilized as recipients of lymphoid cells for each treatment.
† As compared to PLN index of NGS treated lymph-node cells.

Like graft-versus-host reactions, mixed lymphocyte reactions are most readily elicited in other species by allogeneic disparities mapping to the MHC (Klein 1975). Previous studies have demonstrated that hamster strains differing at the putative hamster MHC (*Hm-1*) readily generate strong MLR (Duncan and Streilein 1978 a, 1978 b). Participation of hamster T cells in mixed lymphocyte reactions was studied by mixing GαHT-treated MHA strain lymphoid cells (as responder cells) with irradiated (CB × MHA)F_1 hybrid lymphoid cells (as stimulator cells) in typical MLR culture conditions. Responder MHA strain lymphoid cells were treated with NGS as control. As the results presented in Table 4 indicate, GαHT treatment of responder cells abolishes a significant portion of the hamster mixed lymphocyte reaction. The response was reduced by 58 percent, compared to that of NGS-treated responder cells (stimulation indices of 3.3 vs. 7.9, respectively). Therefore, two different assays of hamster alloimmune reactivity (GVHR and MLR), presumably resulting from disparities encoded by the hamster MHC, require T cells for their induction.

D) Role of hamster T cells in virus-induced, cell-mediated cytotoxicity. We have previously shown that hamsters acutely infected with vaccinia virus generate cytotoxic activity which, in many ways, resembles cytotoxic T effector cells observed in mice and rats (Nelles and Streilein 1980). Primary footpad swelling after local injection of virus, kinetics of virus-induced, cytotoxic activity, and virus specificity of cytotoxic effector cells are consistent with, but not proof of, a thymus-derived hamster effector cell. To resolve this important matter, spleen cells obtained from hamsters acutely infected with vaccinia virus were treated with either GαHT or NGS. Cells remaining after treatment were assayed for cytotoxic activity in vitro in a ^{51}Cr release assay against virus-infected, syngeneic target cells. It can be seen from Table 5 that the hamster virus-induced cytotoxic activity is *not* T-cell-mediated. At E : T ratios of 13 : 1, NGS- and GαHT-treated cells effect almost equivalent levels of cytotoxic activity (55 and 50 percent specific release, respectively). At E : T ratios of 4 : 1, the cytotoxicity of GαHT-treated spleen cells is significantly enhanced as compared to cells treated with NGS (45 and 28 percent specific release, respectively).

Table 4. Effect of goat anti-hamster thymocyte serum on mixed lymphocyte reaction in syrian hamsters

Stimulator	Responder treatment*	CPM incorporated	S.I.	Percent reduction†
MHA	NGS	819 ± 44	–	–
	GαHT	754 ± 171	–	–
(MHA × CB)F_1	NGS	6449 ± 197	7.9	–
	GαHT	2491 ± 128	3.3	58

* MHA lymph-node cells were treated with antiserum or normal serum in the presence of complement, resuspended to equivalent concentrations, and used as responder cells in mixed lymphocyte reaction versus untreated MHA spleen (control) and (MHA × CB)F_1 spleen (experimental) stimulator cells. Values of cpm incorporated represent the mean ± standard error of the mean of three replicate cultures.

† As compared to stimulation index of NGS treated responder lymph-node cells.

Table 5. Effect of goat anti-hamster thymocyte serum on hamster and mouse virus-induced cytotoxic activity

Effector	Treatment	Target	E:T ratio	Specific release	Percent reduction*
Hamster					
Vaccinia immune	NGS	Vaccinia infected	13:1	55±3	–
			4:1	28±3	–
		Uninfected	13:1	8±2	–
	GαHT	Vaccinia infected	13:1	50±2	8
			4:1	45±1	–†
Nonimmune	–	Vaccinia infected	13:1	14±2	–
			4:1	4±1	–
Mouse					
Vaccinia immune	NGS	Vaccinia infected	40:1	47±3	–
			13:1	30±3	–
	GαHT	Vaccinia infected	40:1	11±2	77
			13:1	5±1	83

Spleen cells were obtained from vaccinia virus immune or nonimmune animals and treated with antiserum or normal serum in the presence of complement. Cells were counted, resuspended to equivalent concentrations and assayed for cytotoxic activity in vitro against vaccinia virus-infected target cells in 16 h (hamster) and 6 h (mouse) ^{51}Cr release assays.

* As compared to NGS treated cells (where applicable).

† In this experiment, a 58 percent increase in cytotoxic activity was actually observed.

Media release (spontaneous) for hamster target cells (infected or uninfected) was 16 percent ^{51}Cr release; for murine targets it was 10 percent ^{51}Cr release.

The failure to inhibit the presumed MHC-determined, virus-induced hamster cytotoxic activity with the GαHT reagent was surprising in view of our results describing the requirements for hamster T cells in GVHR and MLR. Moreover, similarities between the experimental design of our vaccinia virus experiments and the classical studies in mice, in addition to the similarities of hamster and mouse virus-induced cytotoxic activities, forced us to question the validity of the GαHT reagent. To verify its capacity to react with T cells possessing known cytotoxic activity, the GαHT reagent, which cross-reacts with murine T cells (data not shown), was used to deplete cytotoxic T cells from spleen-cell suspensions obtained from mice acutely infected with vaccinia virus. These results are also presented in Table 5. Murine spleen-cell suspensions treated with GαHT lose most of their cytotoxic activity when compared to NGS-treated effector cells. Murine cytotoxic T-cell activity is reduced by GαHT treatment at E:T ratios of 40:1 and 13:1, by 77 and 83 percent, respectively. Therefore, the inability of GαHT treatment to eliminate hamster virus-induced cytotoxic activity is not a result of the reagent's lack of reactivity for a putative subset of hamster T effector cells possessing cytotoxic activity. Instead, we conlcude that at least a major portion of vaccinia virus-induced cytotoxic activity in hamsters is not mediated by thymus-derived lymphocytes in

direct contrast to what is observed in mice and rats (Koszinowski and Ertl 1975, Zinkernagel et al. 1977).

Discussion

Syrian hamsters possess functional thymus-derived cells which are involved in various alloimmune reactions in vivo and in vitro. Utilizing a well-characterized, highly selective, heterologous, anti-hamster T-cell antiserum, (GαHT) we have shown a requirement for hamster T cells in mixed lymphocyte (Table 4) and graft-versus-host reactions (Table 3). Studies previously performed in the prototypic murine system have shown such reactivity to be elicited most effectively by class II (*I* region) alloantigenic disparities encoded by the major histocompatibility complex (Klein 1975). Importantly, murine T cells are required for effective induction of these alloimmune reactions.

The results of experiments described in this report are consistent with the hypothesis that Syrian hamsters possess a major histocompatibility complex which encodes lymphocyte-activating determinants (analogous to the murine *I* region of *H-2*). Furthermore, the activated lymphocytes appear to be thymus-derived. The idea that hamsters possess an MHC comparable to murine *H-2* is futher supported by reports that hamster alloantigens involved in acute skin-graft rejection, graft-versus-host reactions, and mixed lymphocyte reactions are closely linked and under dominant single gene control (Duncan and Streilein 1978 b).

Another characteristic of a major histocompatibility complex is the ability of class I (murine *K* and *D* region) encoded molecules to promote the induction of cytotoxic T cells in animals acutely infected with virus (Zinkernagel 1978). In contrast to skin-graft rejection, MLR, and GVHR, alloantigenic disparity is not required for effective induction of virus-specific, cytotoxic T effector cells. However, in order to demonstrate the capacity of class I molecules to promote the induction of cytotoxic effector cells in this system, it was necessary to show that cytotoxic T effector cells were restricted in their killing potential to those target cells expressing the appropriate virus determined antigen in addition to a class I MHC molecule present on the inducing, virus-infected cell. Under physiologic circumstances, class I molecules of the host appear to function in this restricting manner (Doherty et al. 1976).

Hamsters acutely infected with vaccinia virus generate immune cell-mediated reactivity which in many ways is comparable to virus-induced, T-cell-mediated immunity in mice and rats (Nelles and Streilein 1980, Zinkernagel et al. 1977, Tosolini and Mims 1971). The induction of cytotoxic activity in hamsters following acute vaccinia virus-infection and the kinetics and specificity of such virus-induced, cell-mediated, cytotoxic activity suggested that functional class I MHC molecules were operative in hamsters. The results of that study were, however, complicated by the finding that genetic restriction of cytotoxic activity was not observed when hamster strains were utilized across strong immunogenetic barriers. These newly developed and partially inbred hamster strains possess intense, mutual alloreactivity, including the production of highly cytotoxic alloantisera, suggesting disparities of the class I type (Streilein and Duncan 1979).

At that time, two alternative, and mutually exclusive, interpretations were advanced to explain the absence of hamster genetic restriction in the vaccinia virus-induced, cytotoxic system. In the first case, it was suggested that the hamsters studied displayed polymorphism for class II MHC molecules (as judged by MLR and GVHR) but not class I MHC molecules. As a consequence, the presumed T-cell-mediated cytotoxic activity would appear in the experimental results as though unrestricted. The alternative interpretation advanced was that Syrian hamsters do not, or can not, utilize class I MHC molecules to promote the induction of cytotoxic effector cells following acute infection with vaccinia virus. A further implication of this latter argument is that the effector cell would likely not be of thymic origin.

Results presented in this report are inconsistent with the first hypothesis that most, if not all, of the virus-induced hamster cytotoxic activity appears to be mediated by effector cells of thymic origin. Although it can not be proven unequivocally at this time that genetically restricted, cytotoxic T-cell-mediated activity is totally absent in spleens obtained from virus-infected hamsters, it appears that the vast majority of such virus-induced cytotoxic activity is of nonthymic origin in this species. We therefore currently favor the hypothesis that hamsters fail to utilize, or utilize in mininal fashion, MHC-encoded class I molecules to promote virus-induced cytotoxic activity.

Recent studies in man have demonstrated non-*HLA*-restricted, virus-induced cytotoxic activity which is not mediated by cytotoxic T cells (Perrin et al. 1977). In that study, it was suggested that the failure to elicit cytotoxic T-cell activity in revaccinated humans resulted from differing virus dose and/or route of immunization compared to the murine studies. In contrast, hamsters described in this and a previous report (Nelles and Streilein 1980) were immunized with a dose of vaccinia virus and via a route of immunization comparable to the murine and rat studies demonstrating T-cell-mediated cytotoxic activity (Doherty et al. 1976, Zinkernagel et al. 1977). Therefore, the dose of virus and the route of immunization of virus should have optimized the chances of observing virus-induced, T-cell-mediated cytotoxic activity in hamsters.

It would appear that by the criterion of T-cell-mediated alloimmune reactivities such as GVHR and MLR, hamsters possess a conventional major histocompatibility complex (at least with regards to the portion of the chromosome analogous to murine *I* of *H-2* and human *D/DR* of *HLA*). However, the presence and nature of class I-encoded molecules (analogous to molecular determinants encoded by murine *K/D* and human *A*, *B*, *C*,) remain obscure. While hamster lymphoid cell surfaces display a 45 000 mol. wt. glycoprotein species that associates noncovalently with β_2-microglobulin, there is no evidence of serologically detectable polymorphism for these molecules, despite the fact that we have studied strongly disparate hamsters, whose ancestors were captured over a time span of 40 years and at geographic sites many kilometers apart. Moreover, the results of the studies presented here suggest the possibility that hamsters do not utilize these cell-surface molecules, as other species apparently do, to develop cytotoxic T lymphocytes capable of killing virus-infected target cells.

Studies in mice and other species have found T lymphocytes to comprise a heterogeneous population of cells (Cantor and Boyse 1977). By functional criteria and on the basis of surface markers recognized by alloantisera, T cells mediating

MLR, GVHR, and contact and delayed hypersensitivity constitute one subset, usually displaying the Lyt 1+, 23− surface phenotype. Alternatively, T cells responsible for mediating cytotoxic reactions in vitro and for mediating certain types of suppressor functions have an Lyt 1−, 23+ surface phenotype. At present, reagents capable of identifying comparable cell-surface determinants in hamsters are not available. However, we are accumulating functional data which address the spectrum of T-cell-mediated reactivities in this species. Strong MLR and GVHR in hamsters, inhibitable by a selective goat anti-hamster T-cell serum, suggests the presence of the hamster analogues of Lyt 1+, 23− T cells. In addition, the recent description of conventional contact hypersensitivity to simple haptens in Syrian hamsters supports the notion that Lyt 1+, 23− T cells exist (Streilein et al. 1980, Maguire 1979). By contrast, hamsters fail to generate virus-induced, cytotoxic T cells as reported herein; and in systems designed optimally to induce and detect suppressor T cells in an allogeneic contect, no such suppressor reactivity has been found (Lause and Streilein 1978). Thus, at present, we have no direct evidence to conclude that hamsters possess homologous of the Lyt 1−, 23+ subset of T lymphocytes. We are currently examining other effector systems (e. g., CML) in an effort to document the presence of this subset of T cells in hamsters. In the meantime, we are willing to entertain the possibility that our failure to find these T-cell subsets may not be trivial, and that the apparent lack of polymorphism for class I MHC molecules in this species may be a related phenomenon.

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