Different Forms of Membrane-Associated Herpes Simplex Virus Glycoproteins Induce Functionally Distinct Subsets of Herpes Simplex Virus-Specific Suppressor T Cells

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Previous studies have shown that two types of virus-specific suppressor T cells (T_s) are induced in mice made tolerant with herpes simplex virus (HSV)-infected spleen cells (SC). One type of T_s blocks the afferent phase of the delayed hypersensitivity response to HSV ($T_{s\text{-aff}}$), and the other blocks the efferent or effector phase ($T_{s\text{-eff}}$). In this report we show that the induction requirements for these suppressor populations differ. Injection of SC infected for 6 h with HSV at a multiplicity of infection of 5 or less or treated with heat-inactivated virus induced only $T_{s\text{-aff}}$. Similar results were seen with SC incubated for 90 min in virus-free preparations containing only viral proteins. In contrast, the $T_{s\text{-eff}}$ population was induced only by SC treated for 6 h with infectious HSV at a multiplicity of infection of 10. Collectively, these data indicate that $T_{s\text{-aff}}$ are induced by adsorbed HSV antigens on SC, whereas $T_{s\text{-eff}}$ are induced by nascent HSV antigens expressed on infected SC. In addition to their induction requirements, the two types of regulatory cells differ in their expression of effector function. $T_{s\text{-eff}}$ but not $T_{s\text{-aff}}$ require a cyclophosphamide-sensitive target cell in the immune recipient for suppressor function. The possible identity of this target cell and the significance of the different induction requirements between the two types of T_s are discussed.

Studies over the past several years dealing with tolerance to delayed hypersensitivity (DH) responses have proved fruitful in elucidating how suppressor T cells (T_s) carry out their immunoregulatory functions. These studies have shown that T_s responsible for the tolerant state can be induced not only by soluble antigen but by antigen-coupled lymphoid cells as well (1, 6, 21). In most of these studies the coupled antigen has been a simple hapten. Recently, we have described the induction of tolerance mediated by T_s by using lymphoid cells coupled with a complex antigen, namely herpes simplex virus (HSV) (13). Spleen cells (SC) infected with HSV when injected intravenously (i.v.) into mice were shown to induce tolerance in mice to an HSV-specific DH response (13).

Tolerance of DH to HSV is mediated, at least in part, by two types of virus-specific T_s . Injection (i.v.) of soluble virus results in the induction of afferent-acting T_s ($T_{s\text{-aff}}$), whereas i.v. injection of HSV-infected syngeneic SC induces $T_{s\text{-aff}}$ as well as efferent-acting T_s ($T_{s\text{-eff}}$) (13). Both types of T_s suppress the DH response to HSV, but they act on different limbs of the response. $T_{s\text{-aff}}$ block the early afferent phase of the DH response involving the induction and clonal expansion of DH-mediating T cells. $T_{s\text{-eff}}$, on the other hand, act later in the efferent phase by blocking the expression of primed DH-mediating T cells (13). Both T_s are virus-specific in their action; i.e., T_s raised in mice with HSV-induced tolerance did not affect the DH response to a different virus, vesicular stomatitis virus.

The finding that i.v. injection of soluble virus induced only the HSV-specific $T_{s\text{-aff}}$ population, whereas injection of SC treated in vitro with the same or similar virus preparation induced both HSV-specific $T_{s\text{-aff}}$ and $T_{s\text{-eff}}$, suggested that the induction requirements for the populations differ significantly. In the present study we investigated and compared

the induction requirements for each T_s population by using HSV-infected SC as tolerogen. Our results indicate that $T_{s\text{-aff}}$ are induced by SC which have passively adsorbed HSV-soluble proteins on their membrane. In contrast, $T_{s\text{-eff}}$ appear to be induced only by SC which have been actively infected with HSV and express on their surface integral membrane proteins of viral origin. In addition, we show that $T_{s\text{-aff}}$ and $T_{s\text{-eff}}$ differ in their effector requirements for suppression of DH to HSV. The significance of these findings regarding T_s recognition and induction via cell-associated viral antigens is discussed.

MATERIALS AND METHODS

Mice. Five-week-old female BALB/c mice were purchased from Cumberland View Farms, Cumberland, Tenn.

Virus. HSV type 1 (HSV-1) strain KOS Ts5 was used in all experiments. KOS Ts5 is a temperature-sensitive mutant which replicates normally at 34°C, but at the nonpermissive temperature of 39°C very little infectious virus is produced.

The procedure for growing virus in rabbit skin cells or mouse 3T6 cells (of BALB/c origin) has been described previously (12). Virus was stored at -70°C, and virus titers were determined by plaque assay. For use, vials of virus were thawed and sonicated for two 30-s pulses with a Branson well sonicator at setting 9.

Induction and elicitation of DH to HSV. Mice were immunized by subcutaneous HSV injection at four sites on the back. Each mouse received a total of 4×10^6 PFU/0.4-ml volume. Six days later, the mice were ear challenged by the injection of a 10- μ l volume containing 10^5 to 10^6 PFU of heat-inactivated virus into the dorsal side of each ear with a 0.5-cc insulin syringe fitted with a 28-gauge needle (Becton-Dickinson 8461; Becton Dickinson and Co., Rochelle Park, N.J.). Ear swelling was measured 24 h after challenge with an engineers micrometer (Mitutoyo Mfg. Co., Ltd., Tokyo, Japan). Results are expressed as delta (δ) ear swelling in

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which the swelling in unimmunized controls is subtracted from the values obtained from the immunized groups. Ear swelling of unimmunized mice did not exceed 10^{-3} in. (1 in. = 2.54 cm).

In all experiments mice were immunized with virus grown in rabbit skin cells and ear challenged with virus grown in 3T6 cells. This ensured that the immunity measured was to viral antigens and not to cellular components.

Induction of DH tolerance to HSV. Two forms of virus were used to induce tolerance. One form, referred to as HSV particles, was a sonic extract of HSV-1 (KOS Ts5)-infected rabbit skin cells. A 0.5-ml sample containing 10^7 PFU was injected i.v. into mice.

The second form was SC infected with HSV (HSV-SC). These cells were prepared as previously described (13). Briefly, a single-cell suspension of erythrocyte-free SC was prepared from normal BALB/c mice and suspended in Eagle medium supplemented with 2% fetal calf serum and 0.1 mM arginine. The cells were then infected with HSV-1 (clarified by centrifuging at $350 \times g$ for 10 min) at various multiplicities of infection (MOIs), and the virus was allowed to adsorb for 60 to 90 min at 37°C . After viral adsorption, medium was added to a total volume of 5 ml, and the virus-SC suspension was incubated for an additional 6 h at 34°C . The infected SC were then washed three times in Earle balanced salt solution (EBSS), and 5×10^7 infected cells were injected i.v. into normal syngeneic recipients. In some experiments (see Results), the 6-h incubation at 34°C was omitted.

Preparation of virus-depleted sonic extract. Infected rabbit skin cell sonic extracts were depleted of virus by a modified version of a procedure described by Spear and Roizman (16). Briefly, discontinuous sucrose gradients were formed in a polyallomer tube (13 by 51 mm; Beckman Instruments, Inc., Palo Alto, Calif.) by layering, in order from bottom to top, 2 ml of 50% sucrose (prepared in 0.01 M Tris buffer [pH 7.4]), 6 ml of 40% sucrose, and 3 ml of EBSS containing clarified viral sonic extract. The tube was centrifuged in an SW41 rotor at $85,000 \times g$ for 3 h. Two bands were visible after centrifugation. One band was located at the EBSS-40% sucrose interface and represented membrane material, while the second band at the 40 to 50% sucrose interface represented isolated virions. Naked nucleocapsids pelleted to the bottom of the tube. The uppermost EBSS layer and the membrane band at the EBSS-40% sucrose interface were harvested, pooled, and used to treat SC. This procedure depleted infectious particles by more than 99%. SC were suspended in virus-depleted sonic extract in an equivalent volume which would have given infection at an MOI of 2 based on the original virus titer (before centrifuging).

Transfer of tolerance. Seven days after receiving HSV particles or HSV-SC i.v., donor mice were sacrificed, their spleens and lymph nodes were removed (peripheral and mesenteric), and a single-cell suspension was prepared. A total of 10^8 SC or 10^8 SC and lymph node cells mixed 1:1 were injected i.v. into recipient mice in 0.5 ml. The immune status of recipient mice varied depending on whether T_s -aff or T_s -eff function was being examined. To detect T_s -aff, tolerant donor lymphocytes were transferred into naive recipients which were then immunized with virus. Six days later, recipient mice were ear challenged. For detection of T_s -eff, donor lymphocytes were transferred into recipients which had been immunized 6 days previously with virus. Within 1 h of transfer, these recipients were ear challenged. In some experiments, recipient mice were treated with 200 mg of cyclophosphamide (Mead, Johnson and Co., Evanston, Ind.) per kg of body weight 2 days before immu-

nization with HSV-1 or transfer of tolerant lymphocytes. For all experiments, three to four mice per group were used, and each experiment has been repeated at least two times.

RESULTS

Virus concentration requirements for induction of tolerance and T_s . Previous experiments demonstrated that HSV-SC are capable of inducing tolerance and T_s when injected i.v. into mice. In these experiments, the SC were infected with HSV-1 at an MOI of 10 to 20 (13). To determine the minimum concentration of virus required to induce tolerance, SC were infected for 6 h with HSV-1 at different MOIs and then injected i.v. into normal recipients. Seven days later these mice were immunized with virus, and they were ear challenged 6 days after immunization. Significant tolerance was induced by HSV-SC irrespective of the dose of virus used to infect the cells (Table 1, cf. groups B, C, and D). This suggested that the concentration of infectious virus particles was not the critical variable in producing tolerogenic HSV-SC. This conclusion is supported by the results shown in Table 1 for group E. Here, tolerance in mice was induced with SC which had been cultured for 6 h in the absence of virus, washed, suspended in the viral sonic extract at an MOI of 2, and immediately centrifuged. The DH response of this group was suppressed 79%, indicating that simple mixing of SC with viral sonic extract for a brief period is sufficient to render the SC tolerogenic.

Because i.v. injection of HSV-SC infected at an MOI of 10 to 20 induces both T_s -aff and T_s -eff (13), we asked whether these T_s are induced with SC infected with a lower concentration of virus. Thus, donor mice were injected i.v. with HSV-SC (MOI, 2), and 7 days later 10^8 SC were transferred to recipient mice to determine the presence of T_s -aff and T_s -eff (see Materials and Methods). The results of one such experiment (Table 2) show that these HSV-SC induced T_s -aff but not T_s -eff. Additional titration experiments revealed that induction of T_s -eff required injection of SC infected with a higher concentration of virus. SC infected at an MOI of 10 but not at an MOI of 5 or less induced these late-acting T_s (data not shown).

Requirement of infectious virus for induction of T_s -aff and T_s -eff. In other virus systems, induction of tolerance to DH responses has been shown to be dependent on the infectivity

TABLE 1. Induction of tolerance using SC infected in vitro with various MOIs of HSV

Group	MOI of SC ^a	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	% Suppression ^b
A	0 ^c	73.2 ± 5.0	
B	2	5.6 ± 1.8	92
C	1	18.8 ± 4.0	74
D	0.2	17.4 ± 0.7	76
E	2 (no incubation) ^d	15.2 ± 2.2	79

^a SC were infected with various MOIs of HSV sonic extract for 6 h at 34°C , and 5×10^7 HSV-SC were injected i.v. into mice. Seven days later, the mice were immunized with HSV. Six days after immunization, the mice were ear challenged with HSV, and the 24-h ear swelling response was measured (1 in. = 2.54 cm).

^b Percent suppression was calculated as follows: $[1 - (\Delta \text{ tolerant} / \Delta \text{ immunized})] \times 100$. $P < 0.001$.

^c Positive immunity control. Mice did not receive i.v. HSV-SC but were immunized and ear challenged.

^d As in footnote a, except that SC were cultured for 6 h at 34°C without virus. Cells were then centrifuged and suspended in HSV at an MOI of 2. Cells were then washed, and 5×10^7 cells were injected i.v. into mice.

TABLE 2. Differential induction of afferent- versus efferent-acting T_s

Group	Suppressor cell assay ^a	Transfer from HSV-SC-tolerant donors ^b	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	% Suppression ^c
A	Afferent	— ^d	67.1 $\pm$ 4.0	
B	Afferent	10 ⁸ SC	31.9 $\pm$ 5.2	52
C	Efferent	—	65.9 $\pm$ 6.5	
D	Efferent	10 ⁸ SC	69.0 $\pm$ 2.0	0

^a Recipient mice in the afferent assay were naive. Shortly after the passive transfer of donor SC, mice were immunized with HSV, and they were ear challenged 6 days later. Recipient mice in the efferent assay had been immunized with HSV 6 days previously. Within 1 h of passive transfer, mice were ear challenged (1 in. = 2.54 cm).

^b SC were infected with HSV at an MOI of 2 for 6 h at 34°C. Cells (5×10^7) were then injected i.v. into donor mice. Seven days later, donor SC were passively transferred into recipient mice.

^c See footnote b to Table 1.

^d —, Positive immunity control. Mice were immunized and ear challenged without receiving donor cells.

of the virus used as tolerogen (3). For HSV, our results suggest that infectious virus may not be necessary for inducing tolerance (Table 1) or that a dichotomy exists with respect to the necessity of treating SC with infectious virus for induction of T_s -aff versus T_s -eff. To determine whether infectious virus was required for T_s induction, we injected mice with SC treated with either infectious or heat-inactivated virus at the appropriate MOI. The presence of the two T_s subsets was assayed by passive transfer.

SC treated with either infectious or heat-inactivated virus were capable of inducing T_s -aff in the donor mice (Table 3, experiment 1). Suppression of the ear swelling response in mice receiving T_s -aff induced by either form of tolerogen was comparable (cf. groups B and C). In contrast, T_s -eff were induced only by SC treated with infectious virus (Table 3, experiment 2, cf. groups B and C). These results suggest that T_s -aff are induced by viral proteins which are passively adsorbed to SC membranes, whereas T_s -eff are induced by SC which are actively infected with HSV and may be recognizing nascent viral glycoproteins expressed on the infected cell membrane.

SC treated with viral sonic extract depleted of infectious virus can induce T_s -aff. The finding that T_s -aff are induced by SC treated with heat-inactivated virus suggested that viral antigens other than nascently synthesized viral glycopro-

TABLE 4. Induction of T_s -aff by using SC treated with virus-depleted sonic extract

Group	SC treated with: ^a	MOI	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	% Suppression ^b
A	— ^c		63.7 $\pm$ 2.1	
B	Whole sonic extract	2	22.2 $\pm$ 3.5	65
C	Virus-depleted sonic extract	0.02	20.9 $\pm$ 3.3	67

^a SC were treated for 60 min at 37°C with HSV sonic extract (MOI, 2) or sonic extract depleted of infectious virus. After being washed, 5×10^7 treated SC were injected i.v. into donor mice. Seven days later, 10⁸ SC from donor mice were transferred i.v. into recipient mice. Shortly thereafter, recipients were immunized with HSV. Six days later, recipients were ear challenged (1 in. = 2.54 cm).

^b See footnote b to Table 1.

^c See footnote d to Table 2.

teins were associating with SC and rendering them tolerogenic. Because the HSV-infected rabbit skin cell sonic extract used to treat SC contained not only infectious virus but also soluble viral proteins and membrane fragments of infected cells, it was possible that adsorption of these elements to SC was responsible for induction of T_s -aff.

To explore this possibility, we treated SC with virus sonic extract which had been depleted of infectious virus by ultracentrifugation (see Materials and Methods). This supernatant contained less than 1% of the original input virus, and this residual virus accounted for an MOI of 0.02. After incubation with this virus-depleted supernatant, the SC were tested for their ability to induce T_s -aff. SC treated with this virus-depleted supernatant were able to induce T_s -aff in donor mice (Table 4). Suppression of the ear swelling response in recipient mice was equivalent to that seen with T_s -aff induced by SC treated with viral sonic extract which was not centrifuged (cf. groups B and C).

Although the ultracentrifuged supernatant still contained residual infectious virus, additional titration experiments have shown that T_s -aff are not induced with SC treated with an MOI of 0.05 or less (data not shown). Thus, it is unlikely that the contaminating virus particles were responsible for induction of the T_s -aff population. Furthermore, when supernatants of sonically treated material completely devoid of infectious virus particles (obtained by repeated ultracentrifugation of virus sonic extract) were used to treat SC, the T_s -aff population was still induced (data not shown).

TABLE 3. Induction of efferent- but not afferent-acting T_s requires infectious virus

Expt	Group	Infectivity of HSV ^a	Suppressor cell assay ^b	Δ Ear swelling (10^{-4} in. $\pm$ SEM)	% Suppression ^c
1	A	— ^b	Afferent	46.9 $\pm$ 4.8	
	B	Infectious	Afferent	15.1 $\pm$ 1.5	68 ^d
	C	Heat-inactivated	Afferent	20.5 $\pm$ 2.9	56 ^d
2	A	—	Efferent	60.6 $\pm$ 6.1	
	B	Infectious	Efferent	37.7 $\pm$ 3.5	38 ^e
	C	Heat-inactivated	Efferent	59.8 $\pm$ 2.0	1

^a Infectious or heat-inactivated HSV sonic extracts were used to treat SC. In experiment 1, SC were treated with HSV sonic extract at an MOI of 2 for 60 min at 37°C but not incubated for 6 h at 34°C. In experiment 2, SC were treated with HSV sonic extract at an MOI of 10 for 6 h at 34°C. After being washed, SC were injected i.v. into donor mice. Seven days later, SC from donor mice were transferred into afferent or efferent suppressor cell assay recipients.

^b See footnote a to Table 2.

^c See footnote b to Table 1 for method of calculation.

^d $P < 0.001$.

^e $P < 0.01$.

TABLE 5. T_s -eff but not T_s -aff require a cyclophosphamide-sensitive cell in the immune recipient for suppressor function

Expt	Suppressor cell assay ^a	Cyclophosphamide pretreatment ^b	Tolerogen ^c	Donor cells transferred	Δ Ear swelling ($\times 10^{-4}$ in. $\pm$ SEM)	% Suppression ^d
1	Afferent	—	HSV particles	None	75.4 $\pm$ 3.1	
		—		10^8 SC	46.2 $\pm$ 7.0	39 ^e
		+		None	70.0 $\pm$ 4.6	
		+		10^8 SC	33.2 $\pm$ 5.4	53 ^e
2	Afferent	—	HSV-SC (MOI = 2)	None	41.5 $\pm$ 5.0	
		—		10^8 SC	15.9 $\pm$ 2.5	62 ^e
		+		None	57.7 $\pm$ 1.6	
		+		10^8 SC	34.6 $\pm$ 3.9	40 ^f
3	Efferent	—	HSV-SC (MOI = 10)	None	44.8 $\pm$ 2.5	
		—		10^8 SC + lymph node cells	26.0 $\pm$ 2.1	42 ^e
		+		None	40.9 $\pm$ 4.6	
		+		10^8 SC + lymph node cells	40.1 $\pm$ 2.8	2

^a See footnote a to Table 2.^b Recipient mice received 200 mg of cyclophosphamide per mg of body weight before immunization.^c To induce T_s -aff, donor mice were injected i.v. with either 10^7 PFU HSV particles or 5×10^7 HSV SC (MOI, 2). T_s -eff were induced by i.v. HSV SC (MOI, 10). Seven days later, the presence of T_s -aff or T_s -eff in SC and lymph node cells of donor mice was assayed by passive transfer.^d See footnote b to Table 1 for method of calculation.^e $P < 0.001$.^f $P < 0.01$.

Collectively, these results indicate that soluble HSV proteins and perhaps membrane fragments expressing viral glycoproteins which nonspecifically adsorb to SC membranes are responsible for inducing T_s -aff.

T_s -eff require a cyclophosphamide-sensitive cell in the immune recipient for suppressor function. T_s -eff in other suppressor cell systems have been shown to require a cyclophosphamide-sensitive T cell in the immune recipient for demonstration of suppression (15, 17–19). This cyclophosphamide-sensitive T cell arises as a consequence of immunization and has been termed T_s -aux (18) or T_s -3 (15, 17, 19). To determine whether T_s -aff or T_s -eff in the HSV suppressor cell system require such a cyclophosphamide-sensitive cell, both populations of T_s were assayed by passive transfer in recipients which had been pretreated with 200 mg of cyclophosphamide per kg of body weight 2 days before immunization with virus. T_s -aff were induced in donor mice either by i.v. injection of HSV particles or HSV-SC (MOI, 2), while T_s -eff were induced by i.v. HSV-SC (MOI, 10).

T_s -aff induced by two forms of tolerogen were capable of suppressing the ear swelling response in cyclophosphamide-pretreated recipients (Table 5, experiments 1 and 2). In contrast, when T_s -eff were passively transferred into cyclophosphamide-pretreated recipients, no suppression of ear swelling occurred (experiment 3). This indicates that HSV-specific T_s -eff, like T_s -eff in other antigen systems, act through a cyclophosphamide-sensitive cell in the immune recipient to mediate suppression of the DH response. HSV-specific T_s -aff do not require such a cell for suppression.

DISCUSSION

We showed previously that SC infected with HSV when injected i.v. into mice give rise to two types of suppressor cells, T_s -aff and T_s -eff, which suppress the DH response to HSV (13). Both suppressor cells are T cells, and both are virus specific (i.e., the DH response to an unrelated virus is not inhibited) but not type specific for HSV-1 and HSV type 2 (13). Aside from suppressing the DH response in either the

afferent or efferent phase, very little was known which could distinguish the two populations of T_s .

In this report we have characterized other differences between the two cell populations. Our initial approach involved studying the induction requirements of T_s -aff and T_s -eff by using different preparations of HSV-SC as tolerogen. Because the viral sonic extract used for preparing HSV-SC contained not only infectious viral particles but also soluble viral proteins and cellular and viral membrane fragments expressing viral antigens, it is unclear which of these components was responsible for T_s induction. The experiments presented in this paper indicate that the two T_s populations have different requirements for induction and that the various antigen components found in the viral sonic extract play a critical role in the induction process.

The major difference between the induction of the two T_s populations is the requirement that SC be treated with infectious virus. T_s -aff but not T_s -eff are induced in mice which were made tolerant with SC treated with heat-inactivated virus. Because infectious virus was not necessary, this suggested that the nonspecific adsorption of soluble viral proteins or antigenic membrane fragments onto SC might be responsible for induction of the T_s -aff population. More direct evidence of induction by passively adsorbed viral antigens was obtained by using SC treated with soluble viral proteins prepared by separating sonically treated material over a sucrose gradient. These SC were able to induce T_s -aff as efficiently as SC treated with unseparated sonic extract. Thus, the ability of T_s -aff to be induced by SC treated with heat-inactivated virus or supernatants depleted of infectious virus strongly suggests that passively adsorbed viral antigens are responsible for inducing this T_s population.

Because of the enormous amount of structural and nonstructural proteins that are encoded by HSV-1, it would be difficult to determine which proteins in our infected sonic extract are adsorbing onto SC and inducing T_s -aff. This problem can be approached by adsorbing purified HSV-1 glycoproteins on SC and determining whether tolerance and

T_s are induced by these SC. Our laboratory has shown that one such type-specific glycoprotein, glycoprotein C, was capable of inducing type-specific DH and protective immunity when used to immunize mice (14). Preliminary experiments have shown that purified glycoprotein C is also capable of adsorbing onto SC and inducing tolerance against a glycoprotein C DH response. Whether T_s -aff is induced by glycoprotein C-treated SC is currently being investigated. Thus, the use of purified immunogenic HSV-1 glycoproteins may serve to determine whether envelope glycoproteins are capable of inducing T_s when associated with SC, and if so, which ones are involved.

Compared with T_s -aff, T_s -eff had more stringent requirements for induction. This population required that the inducing SC be treated with infectious virus at the relatively high MOI of 10. This requirement appears rather stringent because SC infected with virus at an MOI of 5 or less did not give rise to the efferent-acting suppressor population. These results suggest that viral glycoproteins synthesized and inserted into the SC membrane as a consequence of viral infection are essential for induction of the T_s -eff population. Superinfecting conditions may be required for the synthesis and expression of enough glycoproteins on infected SC, particularly if a specialized population of SC is involved in the induction process. Evidence for such a specialized cell has recently been obtained; HSV-infected plastic-adherent SC were able to induce T_s -eff, whereas the nonadherent fraction failed to do so (unpublished data). In other systems, specialized antigen-presenting cells for T_s are also plastic adherent as well as radioresistant and Ia positive (20).

The question of whether cell-associated viral antigens arise via active infection or by passive adsorption takes on greater significance when the role of major histocompatibility complex restriction in T_s induction is considered. If T_s -eff induction involves recognition of nascent viral antigens on infected SC, such recognition may be in the context of class I major histocompatibility complex antigens. Similarly, T_s -aff induction may involve recognition of adsorbed antigens which have been processed and presented with class II antigens on antigen-presenting cells. Preliminary experiments from our laboratory indicate that major histocompatibility complex restriction appears to be involved in the induction of both T_s populations. Neither population is induced when allogeneic HSV-SC are used as tolerogen. In contrast, when H-2 congenic combinations are used, major histocompatibility complex identity between inducing HSV-SC and recipient mice is sufficient for the induction of both cell populations. The nature of the restricting element(s) is currently being studied. Results from these experiments may reveal further differences between efferent and afferent-acting T_s and may also elucidate some of the mechanisms of T_s induction.

Afferent and efferent T_s were also shown to differ in their effector requirements. T_s -eff did not suppress when transferred into cyclophosphamide-pretreated HSV-immune recipients, indicating that this T_s population requires a cyclophosphamide-sensitive target cell in the immune recipient to regulate the DH response to HSV. T_s -aff did not show such a requirement, i.e., significant suppression by T_s -aff was seen in both untreated and cyclophosphamide-treated recipients. This difference suggests that T_s -aff do not induce T_s -eff but rather that each population is capable of functioning independently. Cyclophosphamide-sensitive T cells in immune animals which interact with T_s -eff to down regulate DH responses have been described in other experimental systems. This cell has been termed T_s -aux (18) or T_s -3 (15,

17, 19) and has the phenotype $\text{Thy } 1^+$, $\text{Lyt } 1^{-2+}$, I-J^+ (15). It should be noted that T_s -aux and T_s -3 described to date have all been induced by immunization with haptens, whereas our report of a T_s -aux- and T_s -3-like cell is, to our knowledge, the first involving regulation of immunity against an infectious agent.

Induction of tolerance and T_s to viral antigens has been described in other laboratories. In most cases, i.v. injection of purified virus particles or viral sonic extracts was used as tolerogen. With reovirus, for example, i.v. administration of infectious virus induced DH, whereas UV-inactivated virus generated T_s (3). The association of virus with antigen-presenting cells was postulated to be the determining factor as to whether immunity or suppression was induced. Nash et al. (8, 10) have also shown that i.v. injection of HSV sonic extract induces tolerance to DH in mice. In their system, UV-inactivated and Formalin-inactivated virus were both capable of inducing tolerance (10). They further showed that two types of T_s were induced, one bearing the phenotype $\text{Lyt } 1^+2^-$ and other bearing the phenotype $\text{Lyt } 1^{-2+}$ (9). Whether these two T_s are afferent- or efferent-acting was not determined. In our system, i.v. injection of HSV sonic extract or HSV-SC induced the T_s -aff population. Thus far, T_s -aff induced by either tolerogen share identical characteristics, including the phenotype $\text{Lyt } 1^{-2+}$. This suggests that i.v. injections of HSV sonic extract may mimic in vitro treatment of SC with HSV in that passive adsorption of viral antigens on lymphoid cells in vivo may be responsible for T_s -aff induction.

The role of HSV-specific T_s in HSV infections has not been established. Modulation of the protective cellular immune response against HSV infections by T_s in HSV infections may have biological significance in certain clinical situations. For example, HSV viremia which occurs in neonates and immunosuppressed patients (2, 4, 7) may contribute to hyporesponsiveness. Whether circulating infected lymphoid cells play a role in this process is not known. We recently developed a protection model in mice (14) and have shown that T cells from donor mice immunized for DH transfer complete protection to naive recipients against lethal HSV infection. Using this model, we have further demonstrated that the T_s -aff population completely inhibits the induction of these protective T cells while having no effect on antibody formation (11). Thus, whereas injecting virus particles or HSV-SC i.v. into mice may induce several immune mechanisms, e.g., natural killers, cytotoxic T cells, and neutralizing antibody, we find that transfer of SC from i.v.-treated mice to naive recipients does not protect against lethal HSV challenge. Rather, such SC contain T_s which inhibit the protective cell-mediated DH response against HSV. Recent studies indicate that T_s may also prevent some of the harmful immunopathological manifestations attributed to immune T cells. With influenza virus, for example, T_s which regulate DH have been shown to inhibit the development of severe immunopathology in the lungs of infected mice (5). Thus, a more complete understanding of the role of HSV-specific regulatory T cells may provide insights into the treatment of HSV infections.

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LITERATURE CITED

1. Bach, B. A., L. Sherman, B. Benacerraf, and M. I. Greene. 1978. Mechanisms of regulation of cell-mediated immunity. II. Induction and suppression of delayed-type hypersensitivity to azobenzenearsonate-coupled syngeneic cells. *J. Immunol.* **121**:1460–1468.
2. Baker, W. H., A. M. Lawton, and K. McCarthy. 1952. Primary generalized infection caused by herpes simplex virus. *Br. Med. J.* **2**:1334–1336.
3. Greene, M. I., and H. L. Weiner. 1980. Delayed hypersensitivity in mice infected with reovirus. II. Induction of tolerance and suppressor T cells to viral specific gene products. *J. Immunol.* **125**:283–287.
4. Johnson, R. T., and C. A. Mims. 1968. Viral infections of the nervous system. *N. Engl. J. Med.* **278**:23–30.
5. Liew, F. Y., and S. M. Russell. 1983. Inhibition of pathogenic effect of effector T cells by specific suppressor T cells during influenza virus infection in mice. *Nature (London)* **304**:541–543.
6. Miller, S. D., and H. N. Claman. 1976. The induction of hapten-specific T cell tolerance by using hapten-modified lymphoid cells. I. Characteristics of tolerance induction. *J. Immunol.* **117**:1519–1526.
7. Nahmias, A. J., C. Alford, and S. Korones. 1970. Infection of the newborn with herpes virus hominus. *Adv. Pediatr.* **17**:185–226.
8. Nash, A. A., and N. P. N. Ashford. 1982. Split T cell tolerance in herpes simplex virus-infected mice and its implication for antiviral immunity. *Immunology* **45**:761–767.
9. Nash, A. A., and P. G. H. Gell. 1983. Membrane phenotype of murine effector and suppressor T cells involved in delayed hypersensitivity and protective immunity to herpes simplex virus. *Cell. Immunol.* **75**:348–355.
10. Nash, A. A., P. G. H. Gell, and P. Wildy. 1981. Tolerance and immunity in mice infected with herpes simplex virus: simultaneous induction of protective immunity and tolerance to delayed-type hypersensitivity. *Immunology* **43**:153–159.
11. Schrier, R. D., G. Y. Ishioka, L. I. Pizer, and J. W. Moorhead. 1985. Delayed hypersensitivity and immune protection against herpes simplex virus: suppressor T cells that can regulate the induction of delayed hypersensitivity effector T cells also regulate the induction of protective T cells. *J. Immunol.* **134**:2889–2893.
12. Schrier, R. D., L. I. Pizer, and J. W. Moorhead. 1982. Delayed hypersensitivity to herpes simplex virus: murine model. *Infect. Immun.* **35**:566–571.
13. Schrier, R. D., L. I. Pizer, and J. W. Moorhead. 1983. Tolerance and suppression of immunity to herpes simplex virus: different presentations of antigens induce different types of suppressor cells. *Infect. Immun.* **40**:514–522.
14. Schrier, R. D., L. I. Pizer, and J. W. Moorhead. 1983. Type-specific delayed hypersensitivity and protective immunity induced by isolated herpes simplex virus glycoprotein. *J. Immunol.* **130**:1413–1418.
15. Sherr, D. H., and M. E. Dorf. 1982. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XIII. Characterization of a third T cell population involved in suppression of *in vitro* PFC responses. *J. Immunol.* **128**:1261–1266.
16. Spear, P. G., and B. Roizman. 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. *J. Virol.* **9**:143–159.
17. Sunday, M. E., B. Benacerraf, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. *J. Exp. Med.* **153**:811–822.
18. Sy, M.-S., S. D. Miller, J. W. Moorhead, and H. N. Claman. 1979. Active suppression of 1-fluoro-2,4-dinitrobenzene-immune T cells. Requirement for an auxiliary T cell induced by antigen. *J. Exp. Med.* **149**:1197–1207.
19. Sy, M.-S., A. Nisonoff, R. N. Germain, B. Benacerraf, and M. I. Greene. 1981. Antigen- and receptor-driven regulatory mechanisms. VIII. Suppression of idiotype-negative, *p*-azobenzenearsonate-specific T cells results from the interaction of an anti-idiotypic second order T suppressor cell with a cross-reactive-idiotypic-positive, *p*-azobenzenearsonate-primed T cell target. *J. Exp. Med.* **153**:1415–1425.
20. Usui, M., I. Aoki, G. H. Sunshine, and M. E. Dorf. 1984. A role for macrophages in suppressor cell induction. *J. Immunol.* **132**:1728–1734.
21. Weinberger, J. Z., B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. III. Interaction of effector suppressor T cells is restricted by H-2 and Igh-V genes. *J. Exp. Med.* **151**:1413–1423.