Role of Suppressor T Cells in Herpes Simplex Virus-Induced Immune Deviation

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Herpes simplex virus type 1 inoculated into the anterior chamber of the mouse eye induces suppression of anti-herpes simplex virus T-cell-mediated delayed hypersensitivity. This suppression is virus-specific, and mediated by splenic T lymphocytes, and it can be adoptively transferred to naive recipients.

Herpes simplex virus (HSV) type 1 (HSV-1) is a leading cause of corneal disease and blindness. To better understand the role of systemic immune responses to intraocular HSV-1, we have utilized a murine model of herpetic ocular disease induced by inoculation of HSV-1 into the anterior chambers (AC) of the eyes of BALB/c mice. HSV inoculated by this route induces aberrant systemic immune responses termed AC-associated immune deviation. Despite intact or enhanced anti-HSV humoral responses, T-cell-mediated delayed hypersensitivity (DTH) responsiveness to HSV-1 is impaired (10). In addition, the AC route of HSV-1 inoculation produces an ocular infection with a distinctive pathogenesis: inoculated eyes develop keratouveitis with retinal preservation, whereas contralateral uninoculated eyes develop primarily posterior segment disease with total destruction of their retinas within 14 days (J. A. Whittum, J. P. McCulley, J. Y. Niederkorn, and J. W. Streilein, in J. W. Chandler and G. R. O'Connor, ed., *Third International Symposium on Immunology and Immunopathology of the Eye*, in press). We have found that AC inoculation of HSV-1 induces a splenic suppressor T-cell population that can be adoptively transferred to suppress normal DTH responsiveness to HSV-1 delivered subcutaneously (s.c.). Since corneal infection with HSV-1 does not induce posterior segment destruction or systemic suppression of T-cell-mediated responses (2), our findings have important implications for the role of systemic immunity in the pathogenesis of ocular HSV infections.

Six- to eight-week-old female BALB/c (*H-2^d*) mice were obtained from Cumberland View Farms, Clinton, Tenn. HSV-1 strain KOS and rabbit skin cells were obtained originally from L. Pizer (University of Colorado Medical School, Denver). Vesicular stomatitis virus (VSV) (Indiana strain) and Vero cells were kindly provided by D. Griffin (Johns Hopkins University School of Medicine, Baltimore, Md.). HSV-1 was grown and titrated on monolayers of rabbit skin cells or Vero cells in Eagle minimal essential medium containing 1% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), and, in Vero cell cultures, nonessential amino acids (0.1 mM). VSV was propagated and titrated on Vero cells. Cell-free supernatants containing virus were prepared as described by Schrier et al. (5). Mock-infected cell culture supernatants were prepared in a similar manner. Virus preparations were stored at -70°C until use.

To assay for active suppression of DTH, we primed donor mice with HSV-1 (2×10^4 PFU) by the AC or intravenous (i.v.) route (4). Control mice were uninoculated or inoculated with HSV-1 or VSV by the s.c. route. Seven days later these mice were sacrificed, and their spleens were removed. Single cell suspensions were made by gentle teasing of spleens in Hanks balanced salt solution containing 1% fetal bovine serum. After two washes in the same medium, cells from similarly inoculated donors were pooled. Naive recipient BALB/c mice (five mice per group) each received one spleen equivalent (1×10^8 to 2×10^8 cells per 0.2 ml) i.v. At 1 to 2 h later, recipients were primed s.c. with 2×10^4 to 4×10^4 PFU of live HSV-1. Six days later, footpads were measured with a Mitutoyo engineer's micrometer, and one hind footpad per mouse was challenged with UV-inactivated (10 min at 12.5 cm from a General Electric G8T5 lamp) HSV-1 (10^6 PFU/0.05 ml) or VSV (5×10^4 PFU). The other footpad received an equal volume of control supernatant. Twenty-four hours later, footpad swelling responses of DTH were measured.

The mean footpad swelling response was determined by the following formula: $\{[\text{experimental (24 - 0 h)} - \text{control (24 - 0 h)}] \times [10^{-4} \text{ in. (} 2.54 \times 10^{-4} \text{ cm)} \pm \text{standard error of the mean}]\}$. Results are expressed as: percent positive = $[\text{experimental (24 - 0 h)} - \text{negative control (24 - 0 h)}] / [\text{positive control (24 - 0 h)} - \text{negative control (24 - 0 h)}] \times 100$.

In some experiments, donor cells were depleted of T cells before transfer by treatment with a monoclonal anti-Thy 1.2 antibody (New England Nuclear Corp., Boston, Mass.) plus complement (GIBCO Laboratories, Grand Island, N.Y.). Briefly, anti-Thy 1.2 antibody (1:500 in Hanks balanced salt solution) was incubated with 50×10^6 cells per ml on ice for 30 min. After removal of the supernatant, an equal volume of complement (1:10) was added, and the mixture was incubated for a further 45 min at 37°C. Cells were washed twice in cold Hanks balanced salt solution containing 1% fetal bovine serum. The remaining cells (one spleen equivalent) were injected i.v. into recipient mice, which were then treated as described above. Control cells were treated with rabbit complement alone. Student's *t* test was used to assess the significance of differences between test groups.

The simultaneous expression of depressed T-cell-mediated DTH and intact (or enhanced) B-cell-mediated (neutralizing-antibody) responses that was described previously and followed AC inoculation of HSV-1 (10) or tumor cells (3, 4, 7, 11) suggested that a regulatory mechanism might be activated during AC-associated immune deviation. To determine whether suppressor cells were induced by the inoculation of HSV-1 into the AC of BALB/c mice, spleen cells

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TABLE 1. T-cell-mediated suppression of DTH responsiveness to HSV-1 after AC or inoculation^a

Expt	Route of donor priming (day -7)	Treatment of donor cells (day 0)	Route of recipient priming (day 0)	Footpad response ($\times 10^{-4}$ in. $\pm$ SEM) (day 7)	% Positive control
A	AC i.v.			32 $\pm$ 11 ^b	0
			s.c.	289 $\pm$ 48	100
			s.c.	87 $\pm$ 20 ^b	21
			s.c.	75 $\pm$ 19 ^b	17
B	AC i.v.	Anti-Thy 1.2 + C C only Anti-Thy 1.2 + C C only		27 $\pm$ 6 ^b	0
			s.c.	245 $\pm$ 44	100
			s.c.	204 $\pm$ 35	81
			s.c.	74 $\pm$ 12 ^b	22
			s.c.	175 $\pm$ 19	68
			s.c.	60 $\pm$ 8 ^b	15

^a Recipient BALB/c mice received spleen cells from donors that had received HSV-1 (2×10^4 to 4×10^4 PFU) by the AC or i.v. route 7 days previously. These cells were treated with antibody plus complement (C) or complement alone immediately before transfer. Recipients were primed s.c. with HSV 1 to 2 h after cell transfer. See text for details of DTH assay and formulas for the calculation of percent positive control.

^b Values were significantly different from those of the positive control group ($P < 0.01$ or better).

were removed from mice that had received HSV-1 by the AC route 7 days previously. The donor cells were inoculated i.v. at a dose of one spleen equivalent per recipient into normal BALB/c mice which, within 2 h, were inoculated s.c. with an immunogenic dose of HSV-1 (2×10^4 PFU). When the footpads of these mice were challenged 6 days later with UV-irradiated HSV-1, only minimal DTH responses were observed (Table 1, experiment A). Footpad swelling responses were comparable for recipients of spleen cells from donors initially inoculated with HSV-1 via the AC or i.v. route. We conclude that an active suppressor mechanism is induced after AC inoculation with HSV-1. These results confirm previous reports of a suppressor population induced by HSV-1 inoculated i.v. (1, 6).

In an effort to characterize the suppressor cells in this system, we next depleted T cells from spleens of mice that had been injected with HSV-1 by the AC route before transfer. Pooled spleen cells from these mice were treated in vitro with anti-Thy 1.2 antibody and complement as described above. (This treatment resulted in a 60 to 65% decrease in total cells recovered.) When this T-cell-depleted population was inoculated i.v. into naive recipients that then received an s.c. injection of HSV-1, normal levels of DTH responsiveness to HSV were observed upon footpad challenge 6 days later (Table 1, experiment B). Spleen cells taken from donors inoculated by the AC route but treated with complement alone suppressed induction of HSV-specific DTH responsiveness in 22% of the positive control group. These experiments demonstrate that suppressor T cells are induced by AC inoculation of HSV-1. In addition, the suppressor cell population induced by i.v. inoculation of HSV-1 was eliminated by anti-Thy 1.2 and complement treatment (Table 1, experiment B, lines 5 and 6). Thus, both the AC and i.v. routes of HSV presentation evoke suppressor T cells that inhibit induction of DTH responsiveness to this virus.

In two experiments, the specificity of suppression induced by AC inoculation of HSV-1 was assessed. We tested the ability of HSV-1-induced suppressor cells to suppress DTH

responses evoked by s.c. immunization of BALB/c mice with VSV. Aliquots of spleen cells from donors that received by the AC route HSV-1 were transferred to naive BALB/c recipients that were then injected s.c. with VSV or HSV-1. The responses of these recipients were compared with those of their respective positive control groups, which had received only s.c. priming with virus. The results of one of these experiments are shown in Table 2. Cells from donors previously infected with HSV-1 by the AC route did not significantly suppress the DTH responses of mice infected with VSV (cf. groups IV and V). Moreover, the transfer of spleen cells from naive BALB/c donors had no effect on the intensity of subsequent recipient DTH responsiveness to HSV-1 after s.c. infection. These results demonstrate that the suppressor cells induced by AC inoculation of HSV-1 are virus specific in their action.

To test further the possibility that the AC and i.v. routes of inoculation of HSV-1 were equivalent, we compared the ability of spleen cells from donors previously injected with HSV-1 by either the AC or the i.v. route to suppress DTH responses of presensitized and naive recipients. We found that adoptive transfer of suppression was possible only when recipient mice received donor cells before the priming dose of virus (data not shown). Thus, both the AC and i.v. priming routes induce suppressor T cells which act at the induction stage, but not at the effector stage, of an anti-HSV DTH response. Whether the suppressor cells are identical or not remains to be determined.

This report describes a suppressor T-cell population induced in spleens of BALB/c mice after HSV-1 inoculation into the AC of the eye. We have demonstrated previously that the AC route of HSV-1 inoculation results in an ocular disease with a distinctive pathogenesis. In association with the developing herpetic ocular infection, deviant systemic immune responses are elicited in which T-cell-mediated anti-HSV DTH responses are impaired despite the induction of vigorous humoral responses to the virus (10). These findings suggested that AC presentation of HSV-1 induces AC-associated immune deviation in a manner similar to that observed after AC inoculation of allogeneic tumor cells (3, 4, 7, 11). The impaired development of T-cell-mediated DTH responsiveness after AC presentation of virus antigens coincides with the induction of suppressor T cells which, upon adoptive transfer, prevent naive animals from developing

TABLE 2. Antigen-specific^a suppression induced by AC inoculation of HSV-1

Group	Route of donor priming (day -7)	Donor-priming virus	Recipient priming virus (day 0)	Elicitation virus	Footpad response ($\times 10^{-4}$ in. $\pm$ SEM) (day 7)	% Positive control
I				HSV	23 $\pm$ 10 ^b	0
II			HSV	HSV	566 $\pm$ 96	100
III	AC	HSV	HSV	HSV	148 $\pm$ 65 ^b	23
IV			VSV	VSV	582 $\pm$ 102	
V	AC	HSV	VSV	VSV	454 $\pm$ 42	
VI	Unprimed		HSV	HSV	503 $\pm$ 99	88

^a Recipient BALB/c mice received spleen cells from donors that had received HSV-1 or no virus 7 days previously. Recipients were primed s.c. 1 to 2 h later with either VSV or HSV-1 (2×10^4 to 4×10^4 PFU). Footpad challenge on day 6 consisted of UV-inactivated VSV (5×10^5 PFU) or HSV-1 (10^6 PFU).

^b Values were significantly different from those of the positive control group (group II; $P < 0.02$); differences between groups IV and V and between groups II and VI were not statistically significant.

DTH responsiveness to HSV. Recently, we have also found that AC-associated immune deviation induced by inoculation of allogeneic tumors into the AC induces suppressor cells that can be revealed similarly by adoptive transfer in vivo (J. Y. Niederkorn, personal communication). Thus, in the HSV system, the allogeneic tumor system, and the hapten-modified spleen cell system (8, 9), AC presentation of antigens to mice activates specific immune suppressor T cells.

AC inoculation of HSV-1 has an effect on the immune system similar, if not identical, to i.v. inoculation of virus. As opposed to the s.c. route of virus inoculation, which regularly leads to the induction of systemic T- and B-cell immunity, the AC and i.v. routes produce specific T-cell nonresponsiveness in DTH reactions that is achieved, at least in part, by suppressor T cells. Yet, via both routes, HSV-1 regularly induces antibody formation. Our findings with regard to the systemic immune responses after AC inoculation of HSV-1 are compatible with the observations of Nash and Ashford (1) and Schrier et al. (5) on systemic immune responses after i.v. inoculation of HSV-1. However, others have reported data which suggest that the AC and i.v. routes are not concordant in systems in which haptenated spleen cells or allogeneic tumor cells are used (4, 8, 9).

Clearly, the AC route of ocular HSV-1 infection differs markedly from the intracorneal route of infection both in terms of the resulting ocular disease and in the nature of systemic immune responses induced. Corneal infections are self-limited and involve only the anterior segment of the eye. Moreover, systemic sensitization to HSV-1 is induced by intracorneal inoculation rather than by the predominance of T-cell-mediated suppression observed after inoculation by the AC route (2). Since the pathogenesis of HSV-induced ocular disease and systemic immune responses after AC inoculation of HSV-1 differs from that of other routes of ocular HSV infection, our findings suggest that the destructive, progressive disease process which follows AC inoculation of HSV may be due to the alteration in systemic immunity. It will be important to determine the relationship between the signal that induces systemic suppression and the distinctive ocular disease that is induced by HSV-1 inoculated by the AC route.

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LITERATURE CITED

1. Nash, A. A., and N. P. N. Ashford. 1982. Split T cell-tolerance in herpes simplex virus-infected mice and its implications for viral immunity. *Immunology* **45**:761-767.
2. Nesburn, A. B. 1981. Immunological aspects of ocular herpes simplex disease. *Proceedings of immunology of the eye; workshop III. Immunol. Abstr. Special Suppl.*, p. 21-42.
3. Niederkorn, J. Y., and J. W. Streilein. 1982. Analysis of antibody production induced by allogeneic tumor cells inoculated into the anterior chamber of the eye. *Transplantation* **33**:573-577.
4. Niederkorn, J. Y., J. W. Streilein, and J. A. Shadduck. 1981. Deviant immune responses to allogeneic tumors injected intracamerally and subcutaneously in mice. *Invest. Ophthalmol. Visual Sci.* **20**:355-363.
5. Schrier, R. D., L. I. Pizer, and J. W. Moorhead. 1982. Delayed hypersensitivity to herpes simplex virus: murine model. *Infect. Immun.* **35**:566-571.
6. 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.
7. Streilein, J. W., J. Y. Niederkorn, and J. A. Shadduck. 1981. Systemic immune unresponsiveness induced in adult mice by anterior chamber presentation of minor histocompatibility antigens. *J. Exp. Med.* **162**:1121-1125.
8. Waldrep, J. C., and H. J. Kaplan. 1983. Hapten-specific unresponsiveness following anterior chamber inoculation. *Invest. Ophthalmol. Visual Sci.* **24**:1339-1345.
9. Wetzig, R. P., C. S. Foster, and M. I. Greene. 1982. Ocular immune responses. I. Priming of A/J mice in the anterior chamber with azobenzenearsonate-derivatized cells induces second-order like suppressor cells. *J. Immunol.* **128**:1753-1757.
10. Whittum, J. A., J. Y. Niederkorn, J. P. McCulley, and J. W. Streilein. 1983. Intracameral inoculation of herpes simplex virus type 1 induces anterior chamber-associated immune deviation. *Curr. Eye Res.* **2**:691-697.
11. Whittum, J. A., J. Y. Niederkorn, and J. W. Streilein. 1982. Alloantigen presentation to the anterior chamber of the eye subverts specific in vitro cell-mediated immune responses. *Transplantation* **34**:190-195.