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B7 Costimulatory Requirements of T Cells at an Inflammatory Site

Haixiao Chen* and Robert L. Hendricks2*†

The requirement for T cell costimulation at sites of infection and inflammation is unresolved. Herpes stromal keratitis (HSK) is a CD4+ T cell-regulated inflammatory response to herpes simplex virus type 1 infection of the cornea. Our findings suggest that susceptibility to HSK is determined by the microenvironment of the infected cornea. The cornea is normally devoid of Langerhans cells (LC), but these APC are present in the surrounding conjunctiva, and migrate into the cornea following infection. The costimulatory molecule B7-2 was constitutively expressed on LC in conjunctiva, but B7-1 was not detectable until 3 days postinfection. LC were the only cells in the cornea that expressed B7-1 through 7 days postinfection. B7-1 was expressed on some, but not all, migrating LC, suggesting that LC migration and B7-1 expression can be independently regulated. The early LC migration and B7-1 expression was independent of T cells, but T cells were required for the massive accumulation of B7-1+ LC in the cornea at the onset of inflammation. Local inhibition of B7-1 function within the infected cornea prevented HSK. Locally blocking B7-2 function did not reduce HSK incidence, but markedly reduce HSK severity. This is the first direct demonstration that naturally expressed B7 is required within an inflammatory site. The Journal of Immunology, 1998, 160: 5045–5052.

The Ag presentation requirements at local sites of infection and inflammation, and the cells that provide this ancillary function, remain poorly defined. The T cell-mediated inflammation that develops in the mouse cornea following herpes simplex virus type 1 (HSV-1)3 infection provides a useful model in which to investigate local APC requirements at an inflammatory site.

In A/J mice, all corneas develop epithelial lesions 2 days after infection with the RE strain of HSV-1. These lesions are caused by virus replication in and destruction of epithelial cells, and are associated with mild and transient inflammatory cell infiltration of the cornea (1). Epithelial lesions heal by 4 days postinfection (p.i.) in both normal and T cell-deficient mice. The corneas then appear normal by both clinical and histopathologic criteria until around day 10 p.i. At that time, about 60% of infected corneas develop an inflammation in the stromal layer that is referred to as herpes stromal keratitis (HSK). It is now well established that HSK in this model represents a CD4+ T cell-regulated inflammatory response in which the Th1-type cytokines IL-2 and IFN-γ play an essential role in regulating neutrophil infiltration and destruction of the cornea (2–6).

Because cytokine transcripts are labile in the absence of T cell stimulation (7), T cells that mediate inflammation require restimulation within the inflamed tissue. Unlike most tissues, the cornea lacks professional APC that are capable of presenting foreign Ags to CD4+ T lymphocytes (8). However, Langerhans cells (LC) are present in the contiguous conjunctival epithelium. Following HSV-1 corneal infection, LC migrate from the conjunctival epithelium into the central cornea (9–11), and corneal LC play an essential role in HSK (9, 12, 13). LC normally reside in tissue as immature cells with limited APC function, but cytokines that are produced by infected tissue, or the infectious agent itself, can stimulate LC migration and maturation (reviewed in Ref. 14). There is evidence that some phenotypic maturation occurs as dermal LC migrate from the skin to the draining lymph nodes (LN) (15). However, such studies are hampered by the difficulty of observing the phenotype of LC within lymphatic vessels. The uniqueness of the cornea lies in the fact that large numbers of migrating LC can be observed, and maturational changes that occur in the LC as they migrate into the central cornea can be readily documented and associated with susceptibility to HSK.

Among the maturational events that enhance the capacity of LC to present Ags to CD4+ T cells is increased expression of the B7 family of costimulatory molecules (16–18). The capacity of B7-1 and B7-2 to deliver a costimulatory signal to T cells by binding to CD28 is well documented (reviewed in Refs. 19 and 20). Systemic treatment with mAb to B7 or with the ligand CTLA4-Ig can reduce the severity of T cell-mediated inflammatory processes, such as experimental autoimmune encephalomyelitis (21–23) and autoimmune diabetes (24, 25). However, the relative role of B7-1 and B7-2 costimulation appears to vary in these two disease models (21–23, 25, 26). Moreover, in these studies it was not clear whether B7 costimulation was required in the inductive phase of the T cell response in the lymphoid organs, in the effector phase of the T cell response in the inflamed tissue, or at both phases of the response. One study suggested that B7-2 costimulation was important in the inductive but not in the effector phase of a hapten-induced contact sensitivity response (27). This conclusion was based on the observation that the contact sensitivity response was reduced by systemic treatment with mAb to B7-2 1 h before sensitization, but was unaffected by similar treatment 1 h before skin challenge. Although the authors’ conclusion is reasonable, it is based on the assumption that systemic mAb treatment 1 h before...

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3 Abbreviations used in this paper: HSV-1, herpes simplex virus type 1; HSK, herpes stromal keratitis; LN, lymph nodes; p.i., postinfection; DTH, delayed-type hypersensitivity; LC, Langerhans cells.

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sensitization affects only the inductive phase of the response, and that systemic mAb treatment 1 h before skin challenge is sufficient to block B7-2 costimulation within the challenge site.

There is evidence for B7 expression at sites of inflammation (28–30), but B7 expression on APCs was not demonstrated, and the requirement for B7 expression within the inflamed tissue was not established. Thus, while blocking B7/CD28 interaction might provide a new avenue of intervention in inflammatory diseases, developing such therapy will require an understanding of the relative role of B7-1 and B7-2 costimulation in a particular disease, and the anatomical site in which costimulation is required. The findings of this study address these important issues in a clinically relevant model of T cell-mediated inflammation.

Materials and Methods

Mice and virus infection

Female A/J mice (Frederick Cancer Research Center, Frederick, MD), 8 to 12 wk old, were anesthetized with 2 mg of ketamine hydrochloride (vetalar; Parke-Davis, Morris Plains, NJ) and 0.04 mg of acepromazine maleate (Aveco, Fort Dodge, IA) in 0.1 ml of HBSS. Topical corneal infection was then achieved by sacrificing the central cornea 10 times with a 30-gauge needle in a crisscross pattern. A 3 µl HSV-1 RE strain virus suspension (5 × 10^5 plaque-forming U) was applied topically to the sacrificed cornea. The HSV-1 RE strain used was propagated on Vero cells and stored at −70°C as previously described (31). All experimental procedures conformed to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research.

Clinical evaluation of HSV-1-infected mice

The severity of HSK was monitored by slit-lamp examination of mouse eyes by an observer who was unaware of the treatment group to which the animal belonged. The degree of stromal inflammation was scored on a scale of 0 to 4+; where 0 indicated no neovascularization and no opacity; 0.5+, slight neovascularization in the peripheral cornea, but no corneal opacity; 1+, neovascularization up to a quarter of the corneal radius, and mild corneal haze; 2+, neovascularization up to three quarters of the corneal radius, and moderate corneal opacity; 3+, neovascularization in the whole cornea with severe opacity, obliterating the view of the iris; and 4+, corneal perforation.

Preparation and staining of corneal epithelial sheets

Infected mouse eyes were enucleated, and epithelial sheets from the cornea and contiguous conjunctiva were separated from the underlying stroma after a 2 h incubation at 37°C in PBS containing 20 mM EDTA. Epithelial sheets were fixed in acetone for 30 min at 4°C and then washed extensively in PBS. For single color staining, the epithelial sheets were incubated overnight at 37°C with primary rat mAb to the DEC-205 Ag on LC (clone 16-10A1), rat mAb to B7-2 (clone GL1), or a combination of both mAbs. Mock-treated controls received similar injections of hamster mAb (anti-dinitrophenyl), clone UC-18B9; American Type Culture Collection (ATCC), Rockville, MD) and rat mAb (anti-HLA-Bw6, clone SFR8-B6; ATCC). Injections were performed with a special apparatus from Hamilton (Reno, NV) that was previously described (32). The injections were given every other day until day 18 p.i. Preliminary experiments established that this treatment resulted in complete blocking of B7-1 or B7-2 expression in the cornea as assessed by immunofluorescent staining with the same mAb that was used to block.

In vivo T cell depletion

To determine whether LC migration and B7-1 expression required a function of T cells, mice were depleted of CD4+ and CD8+ T cells by i.p. injection of a mixture containing 250 µg each of mAb to CD4 (clone OK1.5) and CD8 (clone 2.43). Each mouse received four mAb injections. The first two injections were given at 3-day intervals, and the last two injections were given at 6-day intervals. One cornea of each mouse was infected with HSV-1, and B7-1+ LC were quantified in the corneas 5 days p.i. or 14 days p.i. The timing of the infection was such that both the 5-day p.i. and 14-day p.i. corneas were excised 1 day after the fourth mAb injection. The mAb treatment resulted in at least 98% depletion of CD4+ and CD8+ cells from the LN of randomly selected mice, as assessed by flow cytometric analysis 1 day after the last mAb treatment.

Results

Susceptibility to HSK is not associated with an enhanced systemic Th1 response

HSK develops in about 60% of A/J mice within 14 days after corneal infection. Corneas that are free of inflammation 14 days p.i. do not subsequently develop HSK. Although HSK is a Th1 cytokine-regulated inflammation in HSV-1-infected corneas, susceptibility to HSK was not associated with differences in Th1 cytokine production by HSV Ag-stimulated LN cells, or with the capacity to mount a DTH response to HSV Ags in the skin (Table 1). The DTH response to HSV Ags in this model is mediated by CD4+ T cells, and in part by IFN-γ (33). Thus, following HSV-1 corneal infection, Th1 effector cells are generated in the LN, and can infiltrate and mediate inflammation in infected tissue of mice that do not develop HSK.

Therefore, we hypothesized that susceptibility to HSK might reflect differences in HSV Ag presentation to CD4+ T cells within the cornea. Following HSV-1 infection of the central cornea, LC migrate from the conjunctiva into the central cornea (approximately 2 mm). Our previous study (9) established a necessary role for LC in the development of HSK. Therefore, we determined whether

ELISA

Ninety-six-well plates were coated overnight with primary anti-cytokine capture Ab (4 µg/ml). The plates were washed twice and blocked with 2% BSA in PBS. The supernatant from the LN cell culture and standards were added. After overnight incubation at 4°C, the plates were washed and developed by adding streptavidin-horseradish peroxidase and its substrate. The reaction product was measured with an enzyme immunoassay plate reader at 450 nm. The amount of cytokine in each supernatant was extrapolated from the standard curve. The capture/detection mAbs were as follows: IL-2, JES6-1A12/JES6-5H4; IL-4, 11B11/BVD6-24G2; IFN-γ, R4-6A2/XMG1.2 (all from PharMingen). The sensitivity of detection is 31.3 pg/ml (IL-4) and 50 pg/ml (IL-2 and IFN-γ).

Delayed-type hypersensitivity (DTH) assay

Fourteen days after HSV-1 corneal infection, DTH was elicited by injecting 2 × 10^6 plaque-forming U of UV-inactivated HSV-1 in a volume of 10 µl into the dorsal side of the mouse ear pinna. Ear swelling was measured 24 h later with a Mitutoyo engineers micrometer (Mitutoyo, Tokyo, Japan). The amount of ear swelling (i.e., postchallenge minus prechallenge ear thickness) in HSV-1-infected mice was compared with that of similarly challenged but nonimmunized mice.

In vivo blocking of B7

Beginning 4 days after HSV-1 corneal infection, groups of 12 mice received subconjunctival injections (50 µg in 14 µl) of hamster mAb to B7-1 (clone 16-10A1), rat mAb to B7-2 (clone GL1), or a combination of both mAbs. Mock-treated controls received similar injections of hamster mAb (anti-dinitrophenyl), clone UC-18B9; American Type Culture Collection (ATCC), Rockville, MD) and rat mAb (anti-HLA-Bw6, clone SFR8-B6; ATCC). Injections were performed with a special apparatus from Hamilton (Reno, NV) that was previously described (32). The injections were given every other day until day 18 p.i. Preliminary experiments established that this treatment resulted in complete blocking of B7-1 or B7-2 expression in the cornea as assessed by immunofluorescent staining with the same mAb that was used to block.
susceptibility to HSK was associated with qualitative or quantitative differences in corneal LC. Kinetics of LC migration into HSV-1-infected corneas

Groups of mice received corneal infections with HSV-1, and corneas were excised at various days after infection. Flat mounts of epithelial sheets from these corneas were stained with FITC-conjugated mAb to the LC marker DEC-205, and migrating LC were counted by fluorescence microscopy. LC began to migrate into the cornea 3 days after infection, and continued to accumulate in the central cornea through day 21 p.i. (Fig. 1). The number of LC was significantly (p < 0.0001) higher in corneas that developed HSK than in those without HSK. However, there was little variability in the number of migrating LC before disease onset. These findings clearly establish a relationship between LC migration into infected corneas and HSK, but do not establish the kinetics of LC migration as a predisposing factor for HSK.

B7 expression on LC

The HSK model offered an opportunity to observe the relationship between LC migration and regulation of B7 expression. B7-1 and B7-2 were quantified in flat mounts of corneal/conjunctival epithelium obtained at various times after infection. Our analysis revealed that B7-2 was constitutively expressed on LC within the conjunctival epithelium of normal eyes (Fig. 2A), and was uniformly expressed on LC that migrated into the cornea after infection (Fig. 2B). Thus, there was no obvious relationship between LC migration and B7-2 expression. In contrast, B7-1 was not expressed on LC in the conjunctiva of normal eyes (not shown) or infected eyes (Fig. 3, A and B). However, B7-1 expression was up-regulated on migrating LC in the cornea as early as 3 days after infection. It is noteworthy that not

![Figure 1](http://www.jimmunol.org)  
**FIGURE 1.** Kinetics of LC migration into HSV-1-infected corneas. At various times after corneal infection, corneal inflammation in randomly selected mice was evaluated by slit-lamp examination. The corneas and some contiguous conjunctiva were then excised, and LC were identified in epithelial sheets by immunofluorescent staining with a LC-specific mAb (NLDC-145). The data are recorded as the total number of LC that infiltrated each cornea at a particular time after infection. The dashed line separates the values obtained for corneas with and without HSK. The experiment was repeated twice with similar results.

![Figure 2](http://www.jimmunol.org)  
**FIGURE 2.** B7-2 is constitutively expressed on LC in the conjunctival epithelium of normal eyes. Epithelial sheets were prepared from the cornea and some contiguous conjunctiva of normal mouse eyes (A), and infected eyes with HSK obtained 10 days after HSV-1 corneal infection (B and C). The sheets were stained for B7-2 by indirect immunofluorescent staining with a B7-2-specific mAb (A and B) or an isotype-matched control rat mAb (C). Numerous B7-2+ cells (arrows) with dendritic morphology (inset) are seen in the conjunctival epithelium (A) and corneal epithelium (B) (×132, inset ×330).
all of the migrating LC expressed detectable B7-1 (Fig. 3, C and D). Thus, LC migration and B7-1 expression can be independently regulated. We also observed that through day 7 p.i., all B7-1+ cells in the cornea coexpressed the DEC-205 LC marker. Between days 10 and 21 p.i., corneas with HSK contained B7-1+ cells that exhibited a dendritic morphology and coexpressed DEC-205, and small, round (lymphocyte-like) cells that were negative for DEC-205 (not shown). Between days 14 and 21 p.i., corneas that failed to develop HSK had few, if any, B7-1+ cells. Those that were present exhibited dendritic morphology and coexpressed DEC-205 (not shown).

Figure 4A shows a dichotomy in the number of B7-1+ LC within individual corneas before disease onset. About half of the corneas exhibited elevated numbers of B7-1+ LC at 3 and 7 days p.i., before the onset of HSK. This approximates the frequency of HSK in these mice. Thus, LC expression of B7-1 could be a predisposing factor for HSK. The number of B7-1+ LC in corneas increased dramatically after the onset of HSK (Fig. 4B).

**Are LC migration and B7-1 expression regulated by T cells?**

The initial LC migration and B7-1 expression occurred at 3 days p.i., coincident with active virus replication in the corneal epithelium (days 2 to 5 p.i.). However, the massive accumulation of B7-1+ LC in the infected cornea did not occur until days 10 to 14 p.i., and coincided with the onset of inflammation. We proposed that the early LC migration and B7-1 expression (on day 5 p.i.) was not controlled by T cells, whereas the latter accumulation of B7-1+ LC in the cornea might be T cell dependent. To test this possibility, mice were depleted of CD4+ and CD8+ T cells by i.p. injection of anti-CD4 and anti-CD8 mAb. Corneas of the T cell-depleted or control mAb-treated mice were excised at 5 or 14 days p.i., and B7-1+ LC were quantified within individual corneas. T cell depletion did not influence the number of B7-1+ LC in corneas obtained 5 days p.i., but did significantly reduce the number of B7-1+ LC in corneas obtained 14 days p.i. (Fig. 5). As expected, the corneas of T cell-depleted mice failed to develop inflammation.

**FIGURE 3.** B7-1 is expressed on some migrating LC. Epithelial sheets were obtained from the cornea and some contiguous conjunctiva of 10 eyes 3 days after HSV-1 corneal infection. Using a two-color immunofluorescent staining procedure, B7-1+ cells and DEC-205+ LC were identified within a single field. Nonmigrating DEC-205+ LC in the conjunctiva (A) did not express B7-1 (B). Some migrating DEC-205+ cells in the cornea (C) expressed B7-1, while others were B7-1 negative (D). Total magnification, ×132.

**FIGURE 4.** Kinetics of B7-1 expression on migrating LC. At various times after HSV-1 corneal infection, corneal inflammation in nine randomly selected mice was evaluated by slit-lamp examination. The corneas and some contiguous conjunctiva were then excised, and B7-1-expressing dendritic-shaped cells were identified in epithelial sheets by immunofluorescent staining. Epithelial sheets from normal cornea/conjunctiva were similarly stained. The data are recorded as the number of B7-1+ dendritic-shaped cells in epithelial sheets from corneas with HSK (●) or without HSK (▲). The corneas were obtained before the time of HSK onset (A), or after the time of disease onset (B).
and exhibited a markedly reduced number of B7-1+ LC. Thus, LC migration and B7-1 expression at 14 days p.i. are regulated directly or indirectly by a T cell response in the cornea.

Is B7 costimulation necessary within the infected cornea?

A necessary role for B7 costimulation of CD4+ T cells in the infected cornea was further established by in vivo blocking experiments. Mice received corneal infection with HSV-1 and 4 days later were divided into four treatment groups. Groups of 12 mice received subconjunctival injections of 50 μg of mAb to B7-1, B7-2, B7-1, and B7-2, or control mAb of irrelevant specificity. Injections were initiated on day 4 p.i., and repeated on alternate days through day 18 p.i. Treatment with mAb to B7-1 alone, or a combination treatment with mAb to B7-1 and B7-2 significantly reduced the incidence of HSK in infected corneas (Fig. 6A). In contrast, treatment with mAb to B7-1 did not significantly reduce HSK incidence. However, treatment with mAb to B7-2 did significantly reduce the severity of HSK (Fig. 6B). Thus, both B7-1 and B7-2 costimulation are required for normal progression of T cell-mediated inflammation in HSV-1-infected mouse corneas.

Although the anti-B7-1 and anti-B7-2 mAb were administered locally, and administration was begun 4 days after infection, their effect on HSK could have been exerted in the draining LN rather than in the cornea. To test this possibility, mice received bilateral HSV-1 corneal infections. Four days after infection, a mixture of mAb to B7-1 and B7-2 was administered subconjunctivally to one eye, and control mAb was administered to the companion eye. Injections were repeated and HSK evaluated on alternate days through 18 days p.i. The rationale for this experiment was that the same circulating T cell populations would have access to both the anti-B7 and control mAb-treated corneas. Thus, a normal incidence of HSK in the control mAb-treated eye, but reduced HSK in the anti-B7 mAb-treated companion eye, would establish a requirement for B7 costimulation within the infected cornea.

Following the observation period (on day 19 p.i.), LN-draining eyes that received anti-B7 mAb or control mAb were excised; the LN cells were stimulated with HSV Ags; and their production of IFN-γ, IL-2, and IL-4 was compared. As shown in Table II, these response parameters did not vary significantly when LN-draining anti-B7-treated eyes were compared with those draining control mAb-treated eyes. The fact that local anti-B7 mAb treatment did not influence T cell activation in the LN can be explained in three ways: 1) sufficient numbers of Ag-bearing LC migrated to the LN before mAb treatment to permit optimal T cell activation; 2) LC that migrate from the eye to the LN after anti-B7 treatment might

### Table II.  T lymphocyte response of lymph nodes draining infected corneas that were treated with control mAb or a combination of anti-B7-1 and anti-B7-2 mAb

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control mAb (mean ± SEM)</th>
<th>B7-1 + B7-2 mAb (mean ± SEM)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>3892 ± 1047</td>
<td>4371 ± 1018</td>
<td>p = 0.7479</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>2424 ± 458</td>
<td>2182 ± 281</td>
<td>p = 0.6589</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>77.25 ± 15.81</td>
<td>46.38 ± 6.29</td>
<td>p = 0.0911</td>
</tr>
</tbody>
</table>

* In two experiments, groups of 10 mice received bilateral HSV-1 corneal infection. Beginning on day 4 p.i. and continuing on alternate days thereafter, one eye of each mouse received subconjunctival injections of control mAb, while the companion eye received subconjunctival injections of a cocktail of mAb to B7-1 and B7-2. Preauricular and submandibular LN draining each eye were excised 19 days after HSV-1 corneal infection.

* Cytokine concentrations in supernatants of HSV Ag-stimulated LN cell cultures were determined by ELISA assays.

* Data are for Student’s t test.
Mediated inflammation in HSV-infected corneas. Local blocking of B7 expression prevented the induction of T cell-mediated inflammation in HSV-infected corneas.

Discussion

The effector phase of the T cell response to infectious agents is initiated when infiltrating T cells are restimulated by microbial Ags in infected tissues. It is likely that local specialized APCs are important sources of Ag presentation in these tissues. The uniqueness of the cornea for studying T cell-regulated inflammatory processes derives from the fact that it is normally avascular and lacks professional APCs. We previously demonstrated that LC infiltration into HSV-1-infected corneas was closely associated with CD4+ T cell participation in HSK (9). Therefore, we hypothesized that differences in the susceptibility to HSK of individual HSV-1-infected corneas might be related to the rate of LC migration or maturation. Our findings are not consistent with the notion that the rate of LC migration into the cornea is a predisposing factor for HSK, in that there was little difference in the number of LC in individual corneas before HSK onset.

Resident LC are in an immature state until they are activated by soluble factors that are released in tissues following infection or trauma (reviewed in Ref. 14). In the resting state, LC display low motility and low or no expression of the costimulatory molecules that participate in T cell activation. When exposed to inflammatory cytokines, LC become motile (34, 35) and express phenotypic and functional changes that render them more effective APCs (36, 39), but its role in LC phenotypic maturation is controversial (36, 40). IL-1 and granulocyte-macrophage-CSF up-regulate expression of B7-1 and other costimulatory molecules on LC (16, 17, 36). We observed that depletion of T cells from mice did not affect the number of B7-1+ cells in the cornea 5 days p.i. These observations, coupled with the rapid kinetics of B7-1 up-regulation on migrating LC (within 3 days after infection) are consistent with the notion that LC migration and early up-regulation of B7-1 expression are controlled by cytokines that are produced by corneal cells as a result of HSV-1 infection.

Our studies did not reveal an obvious correlation between susceptibility to HSK and the generation of Th1 cytokine-producing T cells in the LN, or the DTH response to HSV Ags in the skin. It appeared, therefore, that resistance to HSK was probably not associated with 1) reduced generation of Th1 effector cells, 2) reduced capacity of the effector cells to infiltrate infected tissue, or 3) the rate of LC migration into the infected cornea. We did, however, note a correlation between the portion of infected corneas that exhibited elevated numbers of B7-1+ LC before disease onset, and the incidence of HSK. We hypothesize that the density of B7-1+ LC in the cornea at the time of CD4+ T cell infiltration might determine the likelihood that the CD4+ T cells will be stimulated to produce inflammatory cytokines. This possibility was supported by our observations that 1) LC were the only B7-1+ cells in the cornea at the time of HSK onset, and 2) blocking B7-1 prevented HSK.

During the period of 14 to 21 days p.i., corneas that developed HSK had a high density of LC, and most or all of the LC in these corneas were B7-1+. In contrast, corneas that did not develop HSK during this period showed a marked reduction of LC, and few if
any of these LC were B7-1\(^+\). We propose that the massive accumulation of B7-1\(^-\) LC in corneas with HSK is due to the interaction between CD4\(^+\) T cells and the corneal LC. This proposal is supported by our observation that depletion of T cells from mice before HSV-1 corneal infection dramatically reduced the number of B7-1\(^-\) LC in the cornea 14 days p.i. It is well established that interactions between CD4\(^+\) T cells and LC can lead to activation of both the T cell and the LC (reviewed in Ref. 41). For instance, the interaction of CD40 ligand on activated CD4\(^+\) T cells with CD40 on LC induces up-regulation of a variety of costimulatory molecules, including B7-1 by LC, and their production of factors that are chemotactic for LC (18). Such activation might also prevent LC from undergoing apoptotic cell death, which appears to be their ultimate fate (34).

A requisite role for B7 costimulation has been established in several models of inflammation. However, our studies are the first to establish that a T cell-mediated inflammatory response can be regulated by locally blocking B7 costimulation at the inflammatory site. The requirement for costimulation of T effector cells is controversial. It has been suggested that during acute infections, cytokines that are produced by parenchymal or inflammatory cells within the lesion may supplant the need for costimulation of effector T cells (reviewed in Ref. 42). In our model, virus is no longer detectable in the cornea by 5 days p.i., whereas T cell-mediated inflammation is initiated around day 10 p.i. Thus, the initial activation of infiltrating effector T cells occurs in a non-inflamed tissue that lacks replicating virus or immunohistochemically detectable viral Ags. The latter point has led to uncertainties about the Ags that activate the infiltrating CD4\(^+\) T cells. Two possibilities have been proposed. 1) Viral Ags that are processed and presented on conjunctival LC during the period of virus replication are carried by the LC to the central cornea, where they are presented to infiltrating HSV-reactive CD4 T cells. 2) Self-Ags that are released from the immune privileged cornea during virus replication are presented to autoreactive T cells that infiltrate the cornea. In either case, the requirement for costimulation of effector T cells may derive from a combination of weak TCR signaling and the absence of proinflammatory cytokines. Based on the strength of signal hypothesis (43), weak TCR signaling could also account for the preferential involvement of Th1 cytokines in the inflammatory process.

Our findings strongly suggest that susceptibility to HSK is determined by conditions within the microenvironment of the infected cornea. We also establish that B7 costimulation within the cornea is necessary for T cell activation and participation in HSK, suggesting that local manipulation of B7 costimulation might provide an effective means of intervention in this blinding disease.

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