Role of Dendritic Cells in Enhancement of Herpes Simplex Virus Type 1 Latency and Reactivation in Vaccinated Mice

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Ocular infection with herpes simplex virus type 1 (HSV-1) frequently leads to recurrent infection, which is a major cause of corneal scarring. Thus, the prevention of the establishment of latency should be a primary goal of vaccination against HSV-1. To this end, we have examined the contribution of dendritic cells (DCs) to the efficacy of a vaccine against ocular HSV-1 infection. Transgenic mice (expressing a CD11c-diphtheria toxin receptor-green fluorescent protein construct) with a BALB/c background were immunized with a vaccine consisting of DNA that encodes five HSV-1 glycoproteins or were immunized with vector control DNA. The vaccinated mice were then depleted of their DCs through the injection of diphtheria toxin before and after ocular challenge with HSV-1. Analyses of HSV-1 replication in the eye, blepharitis, corneal scarring, and the survival of the infected mice upon primary infection indicated that DC depletion neither promoted nor compromised the efficacy of the vaccine. In contrast, DC depletion was associated with an approximately fivefold reduction in the level of latent virus in the trigeminal ganglia (TGs) of latently infected mice, as well as a significant reduction in the reactivation rate of latent virus. The possibility that DCs enhance the latency of HSV-1 in the TGs of ocularly infected mice suggests for the first time that DCs, rather than acting as "immune saviors," can exacerbate disease and compromise vaccine efficacy by enhancing viral latency and reactivation.

Herpes simplex virus (HSV) infections are among the most frequent serious viral infections in the United States, and it is estimated that 70 to 90% of the adult population in the United States have antibodies to HSV type 1 (HSV-1) and/or HSV-2, with about 25% showing clinical symptoms upon routine medical examination (13, 42). HSV infection can result in virus-induced blindness (5, 72), genital herpes (37), and recurring orolabial lesions (cold sores) (34). During the acute phase of HSV infection, ectodermally derived tissues are commonly involved (15, 59, 67). Early in primary infection, the virus invades local sensory nerves, however, travels via neurons to sensory ganglia, and establishes latency, which persists throughout the lifetime of the infected individual (32, 63). During the life of the latently infected individual, the virus can occasionally reactivate, travel back to the eye, and cause recurrent eye disease (42). Indeed, a major cause of herpes stromal keratitis is the scarring induced by HSV-1 following reactivation from latency (5, 42). HSV-1-induced corneal scarring (CS) can lead to blindness, and HSV-1 is the leading cause of corneal blindness induced by an infectious agent in developed countries (41). In addition, the establishment of latency and the lifelong pattern of episodic recurrence contribute to the spread of the disease in that infected individuals serve as permanent carriers who are intermittently infectious (36, 64).

In individuals with HSV infection, it is the recurrent, rather than the primary, infections that are associated clinically with HSV-induced disease (13, 41). CS is more likely to occur following recurrent, rather than primary, infection (10, 16). Most likely, this pattern reflects the enhancement of the inflammatory conditions that promote CS by the preexisting immune response (36, 64). This issue has two implications for HSV-1 vaccinology. First, the vaccine should be capable of acting therapeutically as well as prophylactically. Second, the stimulation of the various compartments of the immune response during vaccination may lead to inappropriate enhancement of ocular recurrences rather than their alleviation, unless the vaccination strategy is capable of eradicating the virus completely and rapidly. The most efficient way to reduce viral latency and thus subsequent recurrent infections and the loss of vision is both to reduce the virus load and to accelerate virus clearance in the eye and trigeminal ganglia (TGs).

We have shown previously that potent HSV-specific immunity can be achieved by efficient priming of the immune system by DNA vaccines (47, 51). It is thought that DNA vaccines target dendritic cells (DCs) and other antigen-presenting cells (APCs) to induce antigen-specific immune responses in animals. DCs are bone marrow-derived cells, involved in antigen-targeting conditions that promote CS by the preexisting immune response (36, 64). This issue has two implications for HSV-1 vaccinology. First, the vaccine should be capable of acting therapeutically as well as prophylactically. Second, the stimulation of the various compartments of the immune response during vaccination may lead to inappropriate enhancement of ocular recurrences rather than their alleviation, unless the vaccination strategy is capable of eradicating the virus completely and rapidly. The most efficient way to reduce viral latency and thus subsequent recurrent infections and the loss of vision is both to reduce the virus load and to accelerate virus clearance in the eye and trigeminal ganglia (TGs).

We have shown previously that potent HSV-specific immunity can be achieved by efficient priming of the immune system by DNA vaccines (47, 51). It is thought that DNA vaccines target dendritic cells (DCs) and other antigen-presenting cells (APCs) to induce antigen-specific immune responses in animals. DCs are bone marrow-derived cells, involved in antigen capture, processing, and presentation, that trigger the immune response against infectious agents (12, 44, 54, 65, 66, 69). Although both macrophages (6) and DCs (2) can cross present antigens, only DCs are capable of stimulating naïve CD8+ T cells (3, 60). Recently, we have shown that macrophage depletion has a significant effect on reducing vaccine efficacy against ocular HSV-1 replication in the eye and TGs during primary virus replication in immunized mice (47); however, macrophage depletion has no effect on the establishment of latency in infected mice (47). More recently, we have shown that the immunization of BALB/c or C57BL/6 mice with Fms-like tyrosine kinase 3 ligand (Flt3L) DNA, which increases the number of DCs, increases the level of latency in infected mice (49). Conversely, the depletion of DCs is associated with reduced
latency. The results of transfer experiments using DCs expanded ex vivo with Flt3L or granulocyte-macrophage colony-stimulating factor (GM-CSF) suggested that increased latency is associated with the presence of lymphoid (CD11c^+ CD8α^+) DCs and that reduced latency is associated with myeloid (CD11c^- CD8α^-) DCs. However, the modulation of DC numbers by Flt3L DNA immunization or depletion does not alter acute virus replication in the eye or TGs or eye disease in ocularly infected mice. Our results suggest that CD11c^+ CD8α^+ DCs increase the level of HSV-1 latency in mouse TGs. This increase in latency after Flt3L immunization or CD11c^+ CD8α^- transfer is not due to higher-level infection of DCs, since DCs are resistant to HSV-1 replication (1, 49, 61, 68).

In light of the above-described findings, which are contrary to the general beliefs that DCs are the most powerful APCs and that they play a key role in orchestrating the immune response against infectious agents, we undertook studies to determine (i) what role, if any, DCs may play in protection against HSV-1 ocular infection in DNA-vaccinated mice and (ii) whether the depletion of DCs alters the course of HSV-1 infection in ocularly infected mice. Similar to the results in our previous report following ocular infection of naive mice with HSV-1, these results with vaccinated mice suggest that the depletion of DCs does not significantly alter primary virus replication in the eye, HSV-1-induced death, or eye disease in HSV-1-challenged mice. The depletion of the DCs of the DNA-vaccinated mice did, however, lead to a significant reduction in the levels of latent virus in the TGs of the mice that had been challenged with HSV-1, suggesting that some sub-population of the DCs may contribute to the increase of latency.

**MATERIALS AND METHODS**

**Virus, cells, and mice.** Triple-plaque-purified HSV-1 strain McKrae, a stromal-disease-causing and neurovirulent virus, was grown in rabbit skin (RS) cell monolayers in minimal essential medium containing 5% fetal calf serum. Male and female hemizygous C57BL-Tg (Igαx-DTR/GFP) 57Lan1 mice with a BALB/c background were obtained from the Jackson Laboratory (35). Mice were bred at Cedars-Sinai Medical Center and housed in sterile microisolator units. Age- and sex-matched mice used for experiments were between 6 and 8 weeks of age, and all animals were maintained under standard germfree housing conditions at the Cedars-Sinai Medical Center vivarium with the approval of the institutional animal care and use committee.

**Vaccination.** Mice were vaccinated with a mixture of DNA encoding each of the five HSV-1 glycoproteins (gP; gB; gC; gD, gE, and gJ) as described previously (47, 51). In each experiment, mice were inoculated intramuscularly in each quadriceps with a cocktail consisting of 10 μg of cesium chloride-purified DNA for each glycoprotein (50 μg of DNA in a total volume of 100 μl) by using a 27-gauge needle on days 0, 21, and 42. Vector control-vaccinated mice, which were similarly injected with vector DNA alone, served as negative controls. 27-gauge needle on days 0, 21, and 42. Vector control-vaccinated mice, which were similarly injected with vector DNA alone, served as negative controls.

**Depletion of DCs.** BALB/c-DTR mice were depleted of their DCs by using 100 ng of diphtheria toxin (DT) in 100 μl of phosphate-buffered saline administered intraperitoneally as described previously (35), with some modification. Briefly, the first depletion was done 24 h before ocular infection, and this treatment was followed by the second and third depletions 1 and 3 days after ocular infection. Since the treatment of these mice with DT depletes DCs only transiently and DCs begin to repopulate the periphery 48 h after the initial treatment (35), we used multiple depletions corresponding to the duration of virus replication in the eyes of vaccinated mice rather than a single depletion. Therefore, this DT treatment schedule prevented DC repopulation during the course of virus replication in the eyes and TGs of infected mice. Previously, it was reported that repeated DT treatment increases mortality in these mice (75); however, in our hands, fewer than 5% of the depleted mice died as a result of multiple DT treatments. Thus, any infected mouse whose death was not associated with HSV-1 infection was excluded from the survival study. The efficiency of green fluorescent protein-positive CD11c^+ cell depletion in the cornea and spleen was monitored by fluorescence-activated cell sorting (FACS) analysis before ocular infection and 5 days after ocular infection. After the first depletion, the cornea and spleen were depleted of more than 75 and 90% of DCs, respectively. In order to rule out the effect of DT treatment on latency, some wild-type BALB/c mice were injected with DT on a schedule similar to that described above for DTR mice. The effect of DT treatment on latency in latently infected mice was measured. As expected, DT treatment had no effect on latency in wild-type mice (data not shown).

**Ocular infection.** Mice were infected ocularly with 2 x 10^5 PFU of HSV-1 strain McKrae per eye, in 2 μl of tissue culture medium, without corneal scari-lication (27).

**Monitoring eye disease.** The degrees of severity of blepharitis and CS were scored in a masked fashion by examination with a slit lamp biomicroscope following the addition of 1% fluorescein as eye drops. Disease was scored on a scale of 0 to 4 (0, no disease, and 1, 2, 3, and 4, involvement of 25, 50, 75, and 100% of the lid or cornea, respectively) as we described previously (24).

**Monitoring replication and clearance of HSV-1 from the eye.** Monitoring of the replication and clearance of HSV-1 from the eye was performed by swabbing the eyes of a total of 30 mice (60 eyes) in three separate experiments once daily on days 1 to 7 postinfection with a Dacron swab (Spectrum type 1). Each swab was placed in 1 ml of tissue culture medium, and the amount of virus in the medium was determined by a standard plaque assay on RS cells in six-well plates. The plates were incubated at 37°C for 2 days and stained with 1% crystal violet, and the viral plaques were counted.

**In vitro explant reactivation assay.** Mice were sacrificed at 30 days postinfection, and individual TGs were removed and cultured in tissue culture medium. Aliquots of medium were removed from each culture daily for up to 12 days and plated onto indicator cells (RS cells) to look for the appearance of reactivated virus as described previously (53). Since the media from the explanted TG cultures were plated daily, the time at which reactivated virus first appeared in the explanted TG cultures could be determined.

**FACS analysis.** Single-cell suspensions of corneal cells from individual mice were prepared as described previously (27). Single-cell suspensions of spleen cells from each mouse were used as controls. The staining of suspensions was done by incubating cells with monoclonal antibodies (allophycocyanin-conjugated anti-LT4 and phycoerythrin-conjugated anti-Lyt-2 or phycoerythrin-conjugated anti-CD11b) as described by the manufacturer (Pharmingen, San Diego, CA). Single- or double-color flow cytometric analyses of total corneas or spleen cells were performed using a FACScan flow cytometer (Becton Dickinson, PA). The percentages of CD4^+ and CD8^+ T cells or CD11b^+ T cells present were calculated by forward-scatter/side-scatter gating of stained cell populations. For each vaccinated or vector control-vaccinated group, results are represented as the percentage of CD4^+ T cells or CD11b^+ T cells present in the DC-depleted group relative to the number of CD4^+ T cells or CD11b^+ T cells present in the respective mock-depleted group.

**RNA extraction and cDNA synthesis.** Corneas and TGs from vaccinated mice were collected on day 5 postinfection. In addition, to look at the establishment of latency, TGs from some of the surviving mice were collected on day 30 postinfection. The tissues were processed and RNA was isolated as we described previously (48).

**TagMan RT-PCR.** Levels of expression of HSV-1 gB transcripts and latency-associated transcripts (LAT), along with the expression of the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene (used as an endogenous loading control), were evaluated by custom-made TaqMan gene expression assays (Applied Biosystems, Foster City, CA). The gB and LAT primers and probe used were described previously (47, 48). Quantitative real-time PCR (RT-PCR) was performed using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA) with 384-well plates as described previously (47, 48). RT-PCR was performed in triplicate for a given tissue sample from each animal in the group. The threshold cycle (Ct) values, which represent the PCR cycle at which there is a noticeable increase in the reporter fluorescence above the baseline, were determined using SDS 2.2 software. In each experiment, estimated relative numbers of LAT and gB gene copies were calculated using standard curves generated from plasmids containing the gene of interest: pAc-gB and pGem5317. Briefly, each plasmid DNA template was serially diluted 10-fold such that 5 μl contained from 10^3 to 10^5 copies of the desired gene and then subjected to TaqMan PCR with the same set of primers as the test samples. By comparing the normalized Ct of each sample to the Ct of the standards, the copy number for each reaction was determined.
RESULTS

Effect of DC depletion on ocular viral clearance and eye disease in and survival of vaccinated mice. A total of 60 BALB/c-DTR mice (in three separate experiments) were vaccinated three times with 50 μg of 5gP DNA. In addition, a total of 200 mice (in five separate experiments) were vaccinated with the vector DNA alone as a control. Twenty days after the final vaccination and prior to challenge by ocular infection, the sera from 15 5gP DNA-vaccinated and 15 vector DNA control-vaccinated mice were collected. The mean neutralizing antibody titer ± the standard error of the mean (SEM) in the sera of mice vaccinated with 5gP DNA was 672 ± 77, whereas the sera of the vector control-vaccinated mice had a mean titer of 27 ± 11. This difference in neutralizing antibody titers was statistically significant (P < 0.0001; Student’s t test). These results confirmed that the 5gP DNA vaccination induced neutralizing antibody titers in these mice and that the vector control vaccination did not.

On the day of bleeding, one-half of the 5gP DNA-vaccinated mice and one-half of the vector control-vaccinated mice were depleted of their DCs by the administration of DT. Twenty-four hours later, all mice were ocularly infected with 2 × 10^5 PFU of HSV-1 strain McKrae/eye (day 0). The groups of DC-depleted mice were subsequently subjected to repetition of the DC depletion 1 and 3 days after ocular infection with HSV-1. Tear films from 60 eyes/group of the above-described mice were collected daily on days 1 to 7 postinfection, and the amount of virus in each eye was determined by standard plaque assays. In the 5gP DNA-vaccinated mice, DC depletion had no effect on the virus titer or the rapidity of virus clearance compared with that in their mock-depleted counterparts (Fig. 1A). Similarly, the virus titers and the duration of virus clearance in the vector control-vaccinated mice that were depleted of DCs were comparable to those in their mock-depleted counterparts (Fig. 1B). These findings suggest that DC depletion did not result in enhanced HSV-1 replication in the eyes of the vaccinated mice after ocular infection.

Herpetic blepharitis is an inflammation of the lid margin associated with intraocular HSV-1 infection, and in the mouse, increased blepharitis correlates with increased HSV-1 replication (28, 29). Therefore, the eyes of the above-described groups of mice were examined for blepharitis on day 7 postinfection, with the severity of disease being scored on a scale from 0 (no evidence of blepharitis) to 4 (fulminating blepharitis). The average blepharitis score for the mice that had been vaccinated with 5gP DNA and depleted of DCs prior to ocular infection was very similar to the average blepharitis score for their mock-depleted counterparts (Table 1) (P = 0.9; Student’s t test). Similarly, the average blepharitis score for the mice that had been vaccinated with the vector control and depleted of their DCs was very similar to that for their mock-depleted counterparts (Table 1) (P = 0.9). Thus, DC depletion did not have any effect on the severity of blepharitis in the infected mice. As expected, the degrees of blepharitis severity in all of the mice that were vaccinated with 5gP DNA were significantly lower than those in the mice that had been vaccinated with the vector control (Table 1) (P < 0.0001).

Upon examination of the eyes for CS on day 28 postinfection, we found a complete absence of CS in all groups of 5gP DNA-vaccinated mice (score, 0) (Table 1). CS was observed in the mice that had been vaccinated with the vector control, and the vector control-vaccinated mice that had been depleted of DCs had levels of CS similar to those of their mock-depleted counterparts (Table 1) (P = 0.9; Student’s t test). Thus, DC depletion did not appear to alter the levels of CS associated with primary ocular infection.

Finally, the survival of mice in each group following ocular infection with HSV-1 strain McKrae was determined at 30 days postinfection. All (total, n = 30) of the mice that had been vaccinated with 5gP DNA survived ocular infection, whether or not they had been depleted of their DCs (Table 1) (P = 1; Fisher’s exact test). Of the 100 mice that had been vaccinated with the vector control and depleted of their DCs, 24 survived ocular infection, and 19 of the 100 mock-depleted counterparts...
survived; this difference was statistically insignificant (Table 1) (P = 0.5).

Similar results were obtained for mice that were vaccinated with live avirulent HSV-1 strain KOS or a cocktail of 5gP (data not shown). Overall, our results suggest that the depletion of DCs of both 5gP DNA-vaccinated and vector control-vaccinated mice had no effect on virus replication in the eye, blepharitis, CS, or survival after challenge by ocular infection.

**gB and LAT viral mRNAs in the corneas and TGs of depleted mice.** The results of the above-described experiments suggested that the depletion of the DCs did not affect the virus replication in the eyes of the DNA- and vector-vaccinated mice. To further confirm our results presented in Fig. 1, we measured the levels of viral transcripts in the corneas and TGs of infected mice. Mice (10 per group) were vaccinated with 5gP DNA or vector control DNA, and 5 mice from each group were depleted of DCs by using DT or were mock depleted. The mice were then ocularly infected with HSV-1 strain McKrae, and on day 5 after infection, the mice were sacrificed and the corneas and TGs were harvested for the analysis of gB and LAT mRNAs by RT-PCR. In the groups of 5gP DNA-vaccinated mice, the numbers of CD4+ or CD8+ or CD11b+ cells in the corneas or spleen of each mouse were determined using flow cytometry. In the corneas of DC-depleted 5gP DNA-vaccinated mice, the numbers of CD11b+ cells were approximately 2.5-fold higher than those in their mock-depleted counterparts (Fig. 3A). In contrast, the numbers of CD11b+ cells in the spleens of the DC-depleted mice were higher than those in their mock-depleted counterparts whether they had been vaccinated with 5gP DNA or vector control DNA; however, the mice that had been vaccinated with 5gP DNA showed the greatest increase in the numbers of CD11b+ cells upon DC depletion (Fig. 3A).

The numbers of CD4+ T cells in the corneas of the DC-depleted 5gP DNA-vaccinated mice were higher than those in their mock-depleted counterparts (Fig. 3B) (P < 0.05), whereas the numbers of CD8+ T cells in the depleted and mock-depleted 5gP DNA-vaccinated groups were the same (Fig. 3B) (P > 0.05). In the vector DNA-vaccinated mice, DC depletion did not significantly alter the levels of either CD4+ or CD8+ T cells (Fig. 3B) (P > 0.05). The numbers of CD4+ T cells in the spleens of mice in either the 5gP DNA-vaccinated or the vector control-vaccinated group were not affected by DC depletion (Fig. 3B) (P > 0.05). In contrast, the numbers of CD8+ T cells in the spleens of both the 5gP DNA- and the vector control-vaccinated mice were reduced by DC depletion, with the greatest reduction being observed in the vector control-vaccinated mice (Fig. 3B) (P < 0.05).

Thus, DC depletion resulted in greater levels of CD11b+ and CD4+ infiltrates in the corneas of the 5gP DNA-vacci-
nated mice than in those of the vector control-vaccinated group but had no significant effect on the CD8 \(^+\) infiltrates in the corneas. The DC depletion had no effect on the T-cell infiltrates in the spleens of the 5gP DNA-vaccinated mice.

**Effect of DC depletion on establishment of latency.** In neurons, the expression of the more than 80 genes of HSV-1 that are involved in lytic infection is modified drastically during latency. The LAT RNA species is the only gene product that is detected consistently in abundance during latency (17, 21, 33, 58, 67, 71). In general, the level of LAT expression directly correlates with the incidence of recurrences and subsequent induction of eye disease. To determine if DC depletion alters the levels of latency, LAT expression in mouse TGs was measured on day 30 post-ocular infection. TaqMan RT-PCR analysis of the total TG RNA was performed, the level of LAT expression in each group was determined, and the reduction \((n\text{-fold})\) in relation to the level in the mock-depleted counterparts was calculated (Fig. 4). The results indicated that the depletion of DCs in the 5gP DNA-vaccinated group reduced the level of LAT by approximately fivefold in relation to the level in the mock-depleted counterparts (Fig. 4) \((P < 0.01)\). Similarly, the level of LAT in the vector DNA-vaccinated mice that had been depleted of DCs was approximately twofold lower than that in their mock-depleted counterparts (Fig. 4).
Thus, DC depletion significantly reduced the levels of LAT in the TGs of both the 5gP DNA-vaccinated and vector DNA-vaccinated mice.

**Effect of DC depletion on in vitro reactivation of latent virus.** The RT-PCR analyses described above suggested that DC depletion reduced the level of LAT in the TGs of latently infected mice. We therefore investigated whether this reduction in the level of LAT was correlated with a reduction in latent virus reactivation. To accomplish this, the TGs were harvested from the surviving mice from the experiment summarized in Table 1 on day 30 postinfection. The kinetics of virus reactivation in the explanted TGs was then measured. The average reactivation times ± SEM in TGs from 5gP DNA-vaccinated mice were 6.7 ± 0.2 days for the DC-depleted group and 8.9 ± 0.3 days for the mock-depleted counterparts (Fig. 5A) (P < 0.0001; Student’s t test). Similarly, the averages for vector DNA-vaccinated mice were 6.3 ± 0.3 days for the DC-depleted group and 4.0 ± 0.4 days for the mock-depleted counterparts (Fig. 5B) (P = 0.0005). In addition, the RS cells that received the TG aliquots from mock-depleted mice showed faster development of an HSV-1 cytopathic effect (CPE) than RS cells that received TGs from DC-depleted mice (data not shown). These results indicate that DC depletion results in a significant reduction in the reactivation of latent virus due to lower virus loads in the TGs of latently infected mice, which is consistent with the effects of DC depletion on the level of LAT as measured by RT-PCR and shown in Fig. 4.

**DISCUSSION**

Previously, we have shown that both humoral immunity and cell-mediated immunity play a major role in vaccine efficacy against ocular HSV-1 infection (38). We also have shown that the elicitation of neutralizing antibody alone can protect vac-
vincated mice from eye disease and death but does not completely protect the vaccinated mice from virus replication in the eye or the establishment of latency in the TGs (47, 52), suggesting that protection against virus replication in the eye and the establishment of latency is harder to achieve. APCs are known to play a key role in the induction of immunity (12, 44, 54, 57, 65, 66, 69, 74). We recently have shown that the depletion of macrophages in vaccinated mice enhances HSV-1 replication in the eyes of infected mice and is associated with increased blepharitis, although it does not alter the levels of HSV-1-induced death, CS, or latency (47). DCs are considered to be the most potent APCs and to respond rapidly to invading pathogens (12, 54, 56, 69). Because of the critical role that DCs play in orchestrating the immune response via the stimulation of both the innate and adaptive immune systems, there is increasing interest in the use of DCs in cases of transplantation, autoimmunity, chronic inflammation, cancer, and infectious diseases (9, 12, 40). Very little is known regarding whether DCs may be used to improve vaccine efficacy against ocular HSV-1 infection. An improved understanding of the function of DCs in ocular HSV-1 infection may help us to design a vaccination strategy that can reduce or prevent virus replication and the establishment of latency and therefore reduce or eliminate recurrences and eye disease.

In this study, we found that the depletion of DCs had no effect on virus replication in the eyes or TGs of vaccinated mice during the primary phase of HSV-1 infection. The absence of any increase in virus replication in the eyes and TGs of vaccinated mice that had been depleted of their DCs may reflect an increase in the numbers of CD4+ T cells and CD11b+ cells in the corneas of the infected mice. Previously, we have shown that the absence of CD4+ T cells or CD11b+ cells leads to higher levels of virus replication in the eyes of infected mice (25, 47). Although anti-CD11b antibody recognizes the 170-kDa αM subunit of Mac-1 (CD11b/CD18, or αMβ2 integrin) natural killer (NK) cells, we have shown previously that the depletion of NK cells does not increase virus replication in the eyes of immunized mice (26). Furthermore, we recently reported that the immunization of BALB/c and C57BL/6 mice with Flt3L DNA, which increases the number of DCs, increases the level of latency in infected mice (49). Conversely, the depletion of DCs is associated with reduced latency. Latency is also significantly reduced in Flt3L−/− mice, and the immunization of Flt3L−/− mice with Flt3L DNA increases latency. The results of transfer experiments suggested that increased latency is associated with the presence of lymphoid (CD11c+ CD8α−) DCs and that reduced latency is associated with myeloid (CD11c− CD8α−) DCs (49). In the present studies, we have extended our previous findings and found that DC depletion did not alter the incidence or severity of eye disease in the infected mice. It has been reported previously that DCs are required for cross presentation of antigens to T cells (2, 3, 60) and that vaccinia virus abortively infects both mature and immature DCs and blocks their maturation, leading to the impairment of T-cell activation. This discrepancy between our results and those in previously published studies may suggest that other APCs, such as CD11b+ infiltrates, especially macrophages, may compensate for the absence of DCs, as we saw a significant increase in the level of CD11b+ cells in the DC-depleted mice.

In this study, we have found that DC depletion reduced the levels of latency in the TGs of vaccinated and vector-vaccinated mice. In this study, the depletion of DCs from latently infected mice that were immunized with 5gP DNA reduced the level of LAT in TGs by fivefold and, in the control vector-immunized group, the reduction in the level of LAT was two-fold. The smaller twofold reduction may be related to the mouse survival rate of only 20% in the vector-immunized control group. The surviving mice in this group may have persisted because they fell into the low end of the curve for the amount of virus replication in their central nervous systems. This outcome may account for the lower level of LAT in their TGs. This reduction in the establishment of latency in the TGs of the depleted mice was associated with a significant reduction in the reactivation rate of the latent virus in the TGs of depleted mice. Thus, these data indicate that DCs do not play a positive role in improving vaccine efficacy against ocular HSV-1 infection but rather contribute to the increase in latency in the TGs of the infected mice. However, the effect of DC depletion is not unique to the DNA vaccines, since mice that were immunized with the same cocktail of 5gP expressed in baculovirus or immunized with live avirulent HSV-1 strain KOS also showed similar decreases in the levels of LAT (data not shown). Our results cannot be explained by the concept that the depletion of DCs reduces the number of targets of infection with HSV-1, thereby reducing latency in infected mice. It has been shown previously that human blood monocyte-derived DCs are resistant to HSV-1 infection (1, 61, 68), and we have shown that bone marrow-derived DCs isolated from BALB/c mice and cultivated in the presence of Flt3L or GM-CSF are also resistant to HSV-1 replication. In contrast to HSV-1, human immunodeficiency virus (23, 31), dengue virus (73), and vaccinia virus (18) directly target DCs and use them as conduits to increase viral spread by infecting cells with which the DCs come into contact (i.e., T cells).

With HSV-1, DCs are infected but the virus does not replicate in the infected cells. Thus, similar to DCs in human immunodeficiency virus infection, DCs in HSV-1 infection may act as transfer vehicles to spread the virus. Therefore, our results cannot be explained by the concept that the depletion of DCs reduces the number of targets of infection by HSV-1, thereby reducing latency in infected mice. Although in this study we did not find that DCs played a negative role during acute HSV-1 infection, DCs appeared to have a negative impact on HSV-1 infection because they increased latency. The concept that DCs may contribute to the promotion of autoimmune disease has been reported previously (11, 19, 20, 22).

Murine DCs are a heterogeneous population of cells with six known subpopulations that have distinct functions in vivo (8, 62). Recently, we have shown that the transfer of CD11c+ CD8α+ cells to recipient mice that have been depleted of their DCs significantly enhances latency in the TGs of infected mice and that the transfer of CD11c+ CD8α+ cells reduces latency in the TGs. In mice, these two types of DCs have been shown to differ in terms of T-cell stimulation (20, 45, 70), their requirement for different cytokines for the promotion of development (50), their ability to induce TH1 and TH2 responses (46), and their anatomical distribution (43). In addition, it has been reported previously that CD11c+ CD8α+ cells have an inhibitory effect on cytokine production by CD8+ T cells (39),
while other studies have shown that CD11c+ CD8α+ cells are the main subset driving CD8+ T-cell amplification during the early phase of the immune response (7). Previously, it was shown that the exhaustion of CD8+ T cells is the main factor leading to persistent viral infection (4). The CD8+ T-cell impairment is associated with the upregulation of PD-1 (programmed death 1, or CD279) gene expression. In addition, in vivo administration of antibodies that blocks the interaction of this inhibitory receptor with its ligand, PD-L1 (also known as B7-H1, or CD274), enhances T-cell responses and viral clearance (4). Recently, we also have shown that lymphoid (CD11c+ CD8α+) DCs increase the level of HSV-1 latency in mouse TGs (49). Thus, the results of our previous study (49) and our present findings suggest that a certain subpopulation of DCs may directly or indirectly contribute to the impairment of CD8+ T cells and the prevention of viral clearance from TGs, leading to HSV-1 latency. This inhibitory effect of a particular subset of DCs on T cells may be a contributing factor in the ability of DCs to enhance latency in the TGs of the mice in that it may reduce the surveillance capacity of the T cells and compromise their ability to clear infectious virus efficiently, thus enhancing latency without increasing infection. Thus, blocking the interaction of certain DC subpopulations with CD8+ T cells or pushing the maturation of the DCs toward the CD8α+ or CD8α− phenotype may be used as a potentially effective strategy for the enhancement of T-cell responses, and this approach may lead to prevention and/or reduction of latent HSV-1 infection in the TGs. Previously, it was shown that polyethylene glycol-modified GM-CSF expands the CD8α+ population but not the CD8α− population in vivo (14, 55). We are therefore pursuing studies to determine if the inclusion of polyethylene glycol-modified GM-CSF in the 5gP DNA vaccine will reduce latency by pushing the DC subpopulations from a CD8α− to a CD8α+ phenotype.

In summary, our data suggest that (i) DCs do not play a beneficial role in terms of improving vaccine efficacy against ocular HSV-1 infection; (ii) DCs contribute to an increase in latency in HSV-1-infected mice; and (iii) strategies that enhance the activation of DCs in general will not contribute to an improvement of vaccine efficacy against HSV-1 latency, although strategies that enhance the activity of certain populations of DCs may do so.

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ROLE OF DCs IN VACCINE EFFICACY