Overexpression of Interleukin-15 Compromises CD4-Dependent Adaptive Immune Responses against Herpes Simplex Virus 2^†‡

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Interleukin-15 (IL-15) is necessary for the development and function of NK/NKT cells and the maintenance of naive and memory CD8^+ T cells. In the absence of IL-15, protective innate immunity is not available; however, a functional adaptive immune response against vaginal herpes simplex virus 2 (HSV-2) is generated. Mice overexpressing IL-15 (IL-15tg mice) have higher numbers of NK cells, greater NK-derived gamma interferon, and more CD8^+ T cells. Here we examined the consequences of IL-15 overexpression for innate and adaptive immunity against genital HSV-2. Surprisingly, IL-15tg mice immunized against HSV-2 were not protected against genital HSV-2 challenge compared to control immunized mice. IL-15tg mice had a higher frequency of NK cells in the genital mucosa than control mice. However, immunized IL-15tg mice had significantly lower numbers of HSV-2-specific CD4^+ T cells than B6 mice. We then confirmed that CD4^+ T cells, but not CD8^+ T cells, are essential for protection against intravaginal HSV-2 challenge. Since we observed less protection in immunized IL-15tg mice, we then examined if the adaptive immune responses generated in an environment with overexpression of IL-15 could provide protection against HSV-2 in an environment with normal levels of IL-15 expression. We adoptively transferred immunized cells from IL-15tg and B6 mice into naive RAG-1^−/− mice and found that the cells from immunized IL-15tg mice were able to provide protection in this IL-15-normal environment. Our data suggest that overexpression of IL-15 results in a reduced CD4^+ T cell-mediated adaptive immune response against genital HSV-2.

Herpes simplex virus 2 (HSV-2) infection is one of the most widespread infections in the developed world (35). Attempts to develop a vaccine against this viral infection have remained fruitless. Understanding the various factors of the innate and adaptive immune responses that play a role in controlling a herpesvirus infection is essential in designing effective prevention or treatment strategies. Innate immunity is the first line of defense against pathogens, and its role in early control of HSV-2 is well established. Several groups have shown that NK cells, early gamma interferon (IFN-γ) production, and type I IFNs play critical roles in controlling early replication of HSV-2 (1, 2, 5, 13, 18, 19, 32). Recently we have shown that IL-15 also contributes to the innate antiviral responses against herpesvirus (14).

Interleukin 15 (IL-15) belongs to the four-alpha-helix bundle family of cytokines and is similar in structure to IL-2 (8, 15). IL-15 shares the IL-2R β chain and the common γ chain and has its own α receptor for binding and signaling (16). Early studies have shown that IL-15 is essential for the development and activation of NK/NKT cells and secretion of NK cell-derived IFN-γ (16). Furthermore, IL-15 promotes activation of neutrophils and macrophages and is also essential for dendritic cell function. We have recently shown that naive IL-15^−/− mice lacking NK/NKT cells are 100 times more susceptible to HSV-2 infection than normal B6 mice (12). In addition, we have also shown that IL-15 has antiviral properties in the absence of NK/NKT cells (14).

Although a functional innate immune system in necessary for early control of HSV-2, an adaptive response is also crucial for effective clearance of the infection. Initial studies suggested that CD8 T cells were crucial in viral clearance; however, studies in the last several years have highlighted the contribution of CD4 T cells (17, 28–31, 36, 38). In particular, Milligan and Bernstein have shown that CD4 T cell-derived IFN-γ is necessary for protection against HSV-2 infection (30). In our previous study we found that in the absence of IL-15 immunized mice are not protected against genital HSV-2 challenge, although they are able to generate functional adaptive immune responses (12). We concluded that, in the absence of the innate immune response in these mice, the adaptive response is unable to clear the high burden of virus.

IL-15 expression is regulated at the transcriptional level as well as at the translational and intracellular trafficking levels (9). Overexpression of IL-15 leads to an increase in NK cells, NK-derived IFN-γ, and CD8^+ T cells. Mice overexpressing IL-15 (IL-15tg mice) were created by eliminating the normal posttranscriptional control of IL-15, resulting in overexpression of the IL-15 protein (10). These IL-15tg mice were found to have an increase in lymphocytes by 6 to 8 weeks, the major population of these lymphocytes being NK cells (9). There was a decrease in CD4 numbers, while the levels of CD8^+ T cells were increased (9). These mice have been found to be resistant to the development of tumors (41) and show enhanced protection against Mycobacterium bovis, Listeria monocytogenes, Escherichia coli-induced shock, and murine AIDS (20, 39, 40, 42).

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In the present study we hypothesized that overexpression of IL-15, which leads to increased numbers of both NK cells and CD8$^+$ T cells, should result in enhanced protection against vaginal HSV-2 challenge. We first examined the innate and adaptive immune responses against HSV-2 in IL-15tg mice and compared them to those in B6 mice. In contrast to our original hypothesis, overexpression of IL-15 resulted in a lower frequency of HSV-2-specific CD4$^+$ T cells and less protection against genital HSV-2 challenge in immunized mice. We then assessed the role of CD4$^+$ and CD8$^+$ T cells in protection against genital HSV-2 infection. Finally, we evaluated if the HSV-2-specific T cells generated in mice overexpressing IL-15 would provide protection against genital HSV-2 challenge in a normal IL-15 environment.

**MATERIALS AND METHODS**

**Mice.** Female C57BL/6 mice, 8 to 12 weeks old, were purchased from Charles River Laboratory (Québec, Canada). A breeding pair of IL-15tg mice on a C57BL/6 background was kindly provided by M. Caligiuri (Ohio State University, School of Medicine, Columbus). These mice were then bred in the barrier facilities at McMaster University. CD8$^-$ and CD4$^-$ mice on a C57BL/6 background were bred in the Animal Core Facility at McMaster University. RAG1$^{-/-}$ mice, also on a C57BL/6 background, were purchased from Taconic (Germantown, NY). All mice were housed in level B rooms which followed a 12-h day and 12-h night schedule and were maintained under standard temperature-controlled conditions.

**Viruses, cells, and reagents.** HSV-2 strain 333 was grown and titred as previously described (6). Synthetic cytosine-phosphate-guanine (CpG) phosphorothioate oligodeoxynucleotides (ODN) (1826) were purchased from McMaster.

**FIG. 1.** Immunized IL-15tg mice do not survive after IVAG HSV-2 challenge. IL-15tg and B6 mice were intranasally immunized with rgB plus CpG and were boosted 2 weeks later. Two weeks after the booster immunization, mice were treated with medroxyprogesterone acetate and 5 days later were challenged IVAG with $1 \times 10^5$ PFU of HSV-2. Challenged mice were monitored daily for genital pathology and survival. The B6-immunized (imm) mice showed 80% protection, while the IL-15tg-immunized mice showed only 40% survival against HSV-2 challenge. Both IL-15tg naive and B6 naive mice all succumbed to IVAG HSV-2 challenge. This experiment was repeated three times with five mice in each group. A chi-square test was performed and found the survival differences between IL-15tg and B6 mice to be significant ($P < 0.001$).

**FIG. 2.** Innate immune responses in IL-15tg mice are elevated compared to those in B6 mice. (a) Vaginal tissue from HSV-2-infected IL-15tg and B6 (6- to 8-week-old) mice at 24 h postinfection was collected and processed. Cells stained for NK1.1 and analyzed using FACS showed higher percentages of NK1.1-positive cells in IL-15tg mice than in B6 mice. These figures are representative of the staining conducted on six vaginal tissues for each mouse strain. (b) Absolute numbers of NK cells in the vaginal tract postinfection. The asterisk indicates statistical significance ($P < 0.05$). (c and d) Six- to 8-week-old IL-15tg and B6 mice were treated with medroxyprogesterone acetate and then infected with $1 \times 10^5$ PFU of HSV-2. Vaginal washes were collected at days 1 to 3 postinfection and utilized in an IFN-$\gamma$ ELISA, which showed levels of IFN-$\gamma$ in IL-15tg mice (c), or for measuring viral titers at day 1 postinfection, which showed lower viral titers in IL-15tg mice than in B6 mice (d). This experiment was repeated three times with five mice in each group. The asterisk indicates statistical significance ($P < 0.05$).
some experiments, B6 mice were first depleted of CD8+ T cells, each mouse received 200 μg of rgB protein in PBS. In the control group, mice were vaccinated with gB protein only, as described previously (7, 11). The booster immunization preparation included 10^5 PFU/mouse of TK^-minial essential medium supplemented with 1%L-glutamine, penicillin-streptomycin, and 5% fetal bovine serum (Invitrogen, Burlington, ON, Canada). Anti-CD8 antibody (clone 2.43) was prepared by the Centre Core Facility at McMaster University. HSV-2-lysate was prepared by inactivating stock virus with UV light for 30 minutes at 40°C (Stratagene) for the length of time required to prevent viral replication, followed by three rounds of freezing and thawing. Viral inactivation was confirmed by a plaque assay, and the amount of protein was determined. This lysate was used for stimulation of cells in our proliferation assay and intracellular staining protocols.

Immunization. Mice were immunized using thymidine kinase-deficient (TK^-) HSV-2. Briefly, mice were anesthetized and immunized intravaginally (IVAG) with 1 x 10^5 PFU/mouse of TK^-HSV-2 while maintained under anesthetic. In some experiments, B6 mice were first depleted of CD8+ T cells and then immunized. To deplete CD8+ T cells, each mouse received 200 μg of anti-CD8 antibody intraperitoneally at 2 days and 1 day before immunization, and the depletion was maintained by injecting the antibody once a week until the end of the experiment. For survival experiments mice were anesthetized using the gasous anesthetic isoflurane (Bimeda-MTC, Cambridge, ON, Canada) and were immunized intranasally with 15 μl of CpG ODN plus rgB protein. The immunization preparation included 10 μg of CpG ODN and 10 μg of rgB protein in phosphate-buffered saline (PBS). In the control group, mice were vaccinated with gB protein only, as described previously (7, 11). The booster immunization was administered in the same manner 2 weeks after the initial immunization.

Genital HSV-2 inoculation and vaginal virus titration. Mice were injected subcutaneously with 2 mg of medroxyprogesterone acetate/mouse to insure all mice were in a diestrus-like stage (susceptible to HSV-2) before IVAG HSV-2 infection. Five days later mice were anesthetized and infected IVAG with 10^5 PFU of HSV-2 while maintained under anesthetic. B6, IL-15tg, CD8^-/-, and CD4^-/- mice were administered a lethal dose of 1 x 10^6 PFU/mouse, while RAG1^-/- mice were infected with a lethal dose of 1 x 10^5 PFU/mouse. Vaginal washes were collected daily by pipetting twice consecutively 30 μl of PBS into and out of the vagina six to eight times. Viral titers in vaginal washes were determined by plaque assay on a monolayer of Vero cells as previously described (6). Genital pathology was monitored daily after infection. Pathology was scored on a five-point scale as described previously (14).

IgA and IgG antibody ELISA. Genital washes were collected by pipetting twice consecutively 30 μl of PBS into and out of the vagina several times and were stored at ~20°C until use. HSV-2 gB-specific antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA) modified from a protocol described previously (11, 23). Briefly, 96-well Maxisorp plates (Nunc, Roskilde, Denmark) were coated with rgB protein in PBS and incubated overnight at 4°C. After being blocked with 2% bovine serum albumin in PBS, serially diluted samples or controls were added and incubated overnight at 4°C. Biotin-labeled goat anti-mouse immunoglobulin G (IgG) or IgA was added (Pharmingen, Mississauga, ON, Canada), and plates were developed with extravidin-peroxidase (Sigma, St. Louis, MO) and tetramethylbenzidine (KPC, Gaithersburg, MD). Finally, stop solution (1 M H2SO4) was added, and then plates were read with the reader to measure optical density at 450 nm.

Cell proliferation assay. Splenocytes from immunized IL-15tg and C57BL/6 mice were removed, and single-cell suspensions were prepared. Cells were plated at a density of 5 x 10^6 cells/well in 96-well plates. Cells were tested for HSV-2-specific proliferation by addition of HSV-2 lysate (10 μg/ml). Concanavalin A (5 μg/ml; BD Biosciences) was used as a positive control. Cultures were incubated for 48 h, and supernatants were collected and frozen for further testing (IFN-γ ELISA). Proliferative responses were measured by uptake of [3H]thymidine (1 μCi/well) for the last 18 h of a 3-day culture. Results are reported as mean counts per minute of radioactivity ± standard deviations.

ELISA and intracellular cytokine staining (ICC) for IFN-γ. ELISAs for IFN-γ were conducted using the Quantikine murine kit from R&D Systems (Minneapolis, MN). The protocol is a 1-day procedure and was followed according to the manufacturer’s instructions. To detect IFN-γ^-CD4^-/CD8^- T cells, spleen cells from immunized and control B6 and IL-15tg mice were stimulated with HSV-2 lysate (10 μg/ml) or PBS for 6 h and then for an additional 10 h in the presence of GolgiPlug at 37°C, after which the recom-
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FIG. 4. Immunized CD8−/− mice are protected against subsequent HSV-2 infection, while CD4−/− mice succumb to infection. (a) Six- to 8-week-old naive female IL-15tg and B6 mice were sacrificed. Splenocytes were stained for FACS to determine the percentages of CD4 and CD8 cells in these mice. (b) Six- to 8-week-old female CD4−/− CD8−/−, and B6 mice depleted of CD8 T cells were intranasally immunized as described above. Two weeks after the booster immunization, mice were treated with medroxyprogesterone acetate and 5 days later were challenged with 1 × 104 PFU IVAG HSV-2. Mice deficient in CD8 T cells or depleted of CD8 T cells showed survival comparable to that of immunized B6 mice. (c) Immunized CD4−/− mice showed lower percentages of survival than immunized B6 mice. The survival experiments were repeated two times with five or six mice in each group. A chi-square test was performed and found the survival of immunized B6 versus immunized CD4−/− to be statistically significant (P < 0.0001). The FACS figures are representative of the staining of 20 mice in each group.

RESULTS

Immunized IL-15tg mice are not protected against vaginal HSV-2 challenge compared to immunized B6 mice. It is well documented that intranasal immunization provides protection against subsequent IVAG HSV-2 challenge in normal mice (11, 23). We hypothesized that IL-15tg mice, which have greater numbers of NK cells and CD8+ T cells, would be better protected against IVAG challenge after immunization. We challenged immunized IL-15tg and B6 mice with the same challenge dose of 1 × 104 PFU/mouse. Figure 1 shows that, although 80% of the B6 mice were able to survive against challenge, only 40% of the immunized IL-15tg mice were resistant to infection. Furthermore, like naive B6 mice, naive IL-15tg mice all succumbed to infection.

Innate immune responses are heightened in IL-15tg mice compared to B6 control mice. Since, contrary to our hypothesis, immunized IL-15tg mice were less protected against IVAG HSV-2 challenge than B6 mice, we speculated that this may be the result of an improper innate immune response in the genital mucosa. We have previously shown that in the absence of an innate immune response a functional adaptive response is not sufficient to effectively clear the viral infection (12). Hence, we examined whether NK cells in IL-15tg mice are able to home to the vaginal tract following HSV-2 infection or if they
immunized vaginally with TK

from naive B6 mice or naive RAG-1

levels of IFN-

were collected from infected RAG-1

mice ( ) showed 75% protection, while the RAG-1

and the control group, where B6 naive cells were transferred into

immunized IL-15tg mice would be protective in an environment that

protection in immunized IL-15tg mice. We next examined the T-cell responses in these two groups of

adptive transfer of cells from immunized IL-15tg mice into

remain in circulation. Figure 2a shows that the vaginal tracts of

infected IL-15tg mice have a greater percentage of NK cells than those of B6 mice. This increase is quantified in Fig. 2b, which shows that the absolute number of NK cells is significantly increased in the vaginal tracts of IL-15tg mice. Although the NK cells are available at the site of infection, it is necessary to insure that they are functional. Many groups measure the secretion of IFN-γ in the vaginal lumen to assess the activity of innate NK cells. We collected vaginal washes at days 2 and 3 postinfection from B6 and IL-15tg mice to assess the levels of NK cell-derived IFN-γ. Figure 2c shows that the NK cells from IL-15tg mice are functional and produce high levels of IFN-γ in response to infection. Indeed, these innate factors resulted in a significant decrease in viral replication in the first 24 h postinfection in IL-15tg mice compared to replication in B6 mice (Fig. 2d). These results suggest that the innate immune response is available and functional in IL-15tg mice.

Immunized IL-15tg mice have reduced adaptive immune responses compared to immunized B6 mice. Since we found that the vaginal mucosal innate immune responses are not impaired in IL-15tg mice, we then examined the adaptive immune responses generated in these animals following mucosal immunization. Figure 3a shows that gB-specific IgA and IgG levels in immunized IL-15tg and B6 mice are comparable. We next examined the T-cell responses in these two groups of animals. Spleen cells from immunized IL-15tg mice showed lower levels of HSV-2-specific proliferation, as measured by a thymidine incorporation assay (Fig. 3b). This reduced activity was also observed as lower antigen-specific IFN-γ production in immunized IL-15tg animals (Fig. 3c). In order to assess the differences in antigen-specific CD8+ and CD4+ T cells, we utilized ICCS. Figure 3d displays higher absolute numbers of IFN-γ+ CD8+ T cells in immunized IL-15tg mice than in B6 mice, yet a smaller population of IFN-γ+ CD4+ T cells is observed in the IL-15tg mice. These studies demonstrate that there is a difference in the CD8+ and CD4+ T-cell populations in immunized IL-15tg mice.

CD4, but not CD8, T cells are essential for protection against vaginal HSV-2 challenge. Reports have shown that overexpression of IL-15 causes skewing of the lymphocyte population (9). Here we first wanted to examine whether our colony of IL-15tg mice on a B6 background had levels of CD8 and CD4 T cells similar to those of normal B6 mice. Figure 4a illustrates that the overexpression of IL-15 leads to an increase in CD8+ T cells and a decrease in CD4+ T cells compared to levels in normal B6 mice. It has been well established that T cells play a critical role in clearance of genital HSV-2 infection. However, there are conflicting reports in the literature regarding the importance of CD8+ T cells versus CD4+ T cells in providing protection against genital herpesvirus infection (17, 28–31, 36, 38). Here we clarify the role of CD4 and CD8 T cells in this model of mucosal immunization and IVAG challenge. We were able to accomplish this by utilizing mice deficient in CD8 T cells or deficient in CD4 T cells, as well as B6 mice depleted of CD8 T cells in vivo using an anti-CD8 antibody (see Fig. S1 in the supplemental material). We found that immunized CD8−/− mice and B6 mice depleted of CD8+ T cells were both protected against genital HSV-2 challenge (Fig. 4b). However, immunized CD4−/− had only a 20% survival rate after infection (Fig. 4c). These results suggest that CD4 T cells play a more pivotal role in control and/or clearance of HSV-2 in this model and are in agreement with recent reports (17, 30, 36).

Adoptive transfer of cells from immunized IL-15tg mice into naive RAG-1−/− mice provides protection against subsequent vaginal HSV-2 challenge. We confirmed that CD4+ T cells are crucial for protection against IVAG HSV-2 challenge, and we observed significantly lower CD4+ T-cell responses against HSV-2 in IL-15tg mice than in B6 control mice. This led us to hypothesize that these factors may be the reason for reduced protection in immunized mice overexpressing IL-15. We next wanted to determine whether the adaptive immune cells generated in IL-15tg mice would be protective in an environment that had normal levels of IL-15. We accomplished this by transferring...
lymphocytes from immunized IL-15tg and B6 mice into naive RAG-1−/− (RAG1ko) mice that were part of the adoptive transfer study and that were not showing any pathology by day 15 postinfection were sacrificed to examine T-cell responses. (a) IFN-γ levels were measured in supernatants of splenocytes treated with HSV-2 lysate for 48 h. RAG-1−/− mice receiving cells from IL-15tg mice had levels of IFN-γ comparable to those in RAG-1−/− mice receiving cells from immunized B6 mice. (b to e) Surface staining was carried out to determine T-cell populations in the groups of mice. Percentages of CD8+ T cells were lower in RAG-1−/− mice receiving immunized B6 cells (b) than in RAG-1−/− mice receiving immunized IL-15tg cells (c) or B6 naive (d) or IL-15tg naive (e) mice. CD4 T-cell percentages for both groups of RAG-1−/− mice that were part of the adoptive transfer study were comparable (b and c).

**DISCUSSION**

The goal of this study was to understand the role of overexpression of IL-15 on innate and adaptive immunity to genital HSV-2. Although antibody responses in IL-15tg and B6 mice...
were comparable, the T-cell responses differed between the two groups. More specifically, IL-15tg mice have decreased levels of HSV-2-specific CD4+ T cells and CD4+ T cell-derived IFN-γ. We confirmed the importance of CD4+ T cells in the genital HSV-2 model by utilizing immunized CD4+/− mice, which were unable to survive a vaginal HSV-2 challenge, while immunized CD8-deficient mice were protected against infection. Upon further examination we found that the T-cell balance in IL-15tg mice is skewed; however, when these cells are adoptively transferred into a naive RAG1−/− mouse, they are able to confer protection.

Elements conferring innate immunity, in particular type I IFNs, NK cells, IFN-γ, and IL-15, are all necessary for the early control of viral replication (1, 2, 5, 13, 18, 19, 32). Our previous study outlined that, without an adequate innate immune response, a protective adaptive response is not sufficient. However, studies since then have identified CD4+ T cells as the main mediators of protection against HSV-2 in both the genital infection model and the cutaneous zosteriform model (17, 22, 27, 29, 36). Further investigation described the importance of IFN-γ in the adaptive immune response against HSV-2, and the production of this IFN-γ was attributed to CD4+ T cells in the vaginal mucosa (4, 30, 32). Furthermore, it has been shown that in humans enhanced IFN-γ production by CD4+ T cells in response to a particular immediate early protein of HSV-2, ICP4, is correlated with low rate of disease recurrence (4). Our studies found that immunized CD8−/−, or CD8-depleted, mice were protected against subsequent IVAG HSV-2 challenge, while immunized CD4−/− mice succumbed to the subsequent HSV-2 challenge. This confirms the finding of several others that, in the genital HSV-2 model, CD4+ T cells are essential in effectively clearing an HSV-2 infection.

Although the role of IL-15 in innate and/or adaptive protection against HSV-2 has become increasingly evident, there remain questions that have not yet been addressed. We have shown previously that naive or immunized IL-15−/− mice that are deficient in IL-15 and NK/NKT cells are unable to survive vaginal HSV-2 infection (2, 12). However, these mice generate functional adaptive cells, which when transferred are able to protect naive mice that have competent innate immune responses. In the current study we have found that overexpression of IL-15 is also not sufficient for protection against vaginal HSV-2 infection. Our results suggest that the skewing of the T-cell response by IL-15 is responsible for this reduced protection; however, the mechanism by which this is carried out remains elusive.

Several studies have found IL-15 to be important for the maintenance of naive and memory CD8 T cells (3, 9, 25, 26, 34, 43). However, the relationship between IL-15 and CD4+ T cells is not yet well established. Some reports suggest that IL-15 is a negative regulator of CD4+ T cells (24), while others show that IL-15 can enhance CD4 activation in vitro (33, 44). In our study, IL-15tg mice were not protected against HSV-2 infection and showed an increase in CD8− T-cell numbers and a proportional decrease in CD4+ T cells. This raises two main possibilities: (i) an increase in IL-15 drives proliferation of CD8+ T cells to shift the balance of T cells and hence results...
in reduced CD4+ T cells or (ii) IL-15 directly inhibits CD4+ T-cell expansion. A study by Toka and Rouse shows that intranasal delivery of a DNA plasmid encoding the gB protein of HSV-2 along with a plasmid encoding IL-15 enhances CD8+ T-cell responses against HSV-2 in the genital mucosa. They also showed that in this model the mice are protected against subsequent HSV-2 challenge; however, they did not examine the CD4+ T-cell responses in these mice, which in fact may not have been affected by this transient IL-15 presence (37). In our model we have noted that overexpression of IL-15 results in high CD8+ T-cell numbers and lower CD4+ T-cell numbers, which make the mice susceptible to HSV-2 infection. However, this alone does not allow us to understand what role IL-15 has on CD4+ T-cell expansion and function; it only suggests that there is not enough space for both sets of T cells to expand at the same rate, and hence one population dominates. After transferring cells from IL-15tg mice to a naive RAG1−/− mouse, we saw an increase in CD4+ T cells, and the amount of IFN-γ produced by these cells in response to HSV-2 was comparable to that produced by B6 cells in the RAG1−/− mice. This suggests that HSV-2-specific CD4+ T cells are generated in IL-15tg mice and are able to proliferate and elicit effector functions in an IL-15-normal environment.

From these observations we hypothesize that excess IL-15 drives an increase in CD8+ T cells and (i) due to T-cell competition for space and ligands (ii) this reduces the number of effector CD4+ T cells generated. Furthermore, we speculate that IL-15 does not directly impact the generation of effector CD4+ T cells but may inhibit the expansion of such a cell population in addition to driving CD8+ T cells. Findings from a recent study confirm our results that IL-15tg mice have reduced IFN-γ production (21). However Kagimoto et al. attribute the reduction in IFN-γ to an increase in transforming growth factor β, whereas our studies attribute this decrease in IFN-γ to a reduction in CD4+ T cells. Furthermore, we found that after transfer the level of IFN-γ in the vaginal secretions was increased in RAG1 knockout mice receiving IL-15tg immunized cells, however not to the same extent as in RAG1 knockout mice receiving B6 immunized cells. This suggests that at this early time point there may not be as many CD4+ T cells available in the mice receiving IL-15tg lymphocytes as are available in mice receiving B6 lymphocytes, and the inhibitory effects of excess IL-15 may continue to affect these cells even 2 to 3 days posttransfer.

These studies outline the importance of both innate and CD4+ T cell-mediated adaptive immune responses in effective clearance of genital HSV-2 infection. Our previous study demonstrated that a functional adaptive immune response is not sufficient for clearing the viral infection in the absence of an innate immune response (12). In our current study we outlined the importance of an adaptive immune response in effective clearance of HSV-2 as well. Hence, although the IL-15tg mice have higher numbers of NK cells and low viral titers at early times postinfection, they still succumb to infection. These experiments show that, in order to successfully clear a genital HSV-2 infection, there is a requirement for both an innate immune response and a functional and “proper” adaptive response, as depicted in Fig. 8. Our data clearly suggest that in this genital HSV-2 infection model the innate immune system is needed to control HSV-2 early and that clearance of HSV-2 is dependent on HSV-2-specific CD4+ T cells but not CD8+ T cells. Finally, our data provide groundwork for further studies in understanding the role of IL-15 in initiating adaptive immune responses, especially CD4+ T cell-mediated functions.

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REFERENCES

provides various degrees of protection against herpes simplex virus infection. Antivir. Res. 56:39–49.


