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Reduced Frequency of Murine Cytomegalovirus Retinitis in C57BL/6 Mice Correlates with Low Levels of Suppressor of Cytokine Signaling (SOCS)1 and SOCS3 Expression within the Eye during Corticosteroid-Induced Immunosuppression

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Abstract

AIDS-related human cytomegalovirus retinitis remains a leading cause of blindness worldwide. We compared two C57BL/6 mouse models of experimental murine cytomegalovirus (MCMV) retinitis for intraocular expression of suppressors of cytokine signaling (SOCS)1 and SOCS3, host proteins that are inducible negative feedback regulators of cytokine signaling. These mouse models differed in method of immune suppression, one by retrovirus-induced immune suppression (MAIDS) and the other by corticosteroid-induced immune suppression. Following subretinal injection of MCMV to induce retinitis, intraocular SOCS1 and SOCS3 were only mildly stimulated, and often without significance, within MCMV-infected eyes during the progression of MCMV retinitis in corticosteroid-immunosuppressed mice, contrary to MCMV-infected eyes of mice with MAIDS that showed significant high stimulation of SOCS1 and SOCS3 expression in agreement with previous findings. Frequency and severity of retinitis as well as amounts of intraocular infectious MCMV in corticosteroid-immunosuppressed mice were also unexpectedly lower than values previously reported for MAIDS animals during MCMV retinitis. These data reveal a major difference between two mouse models of experimental MCMV retinitis and suggest a possible link between the amplitude of SOCS1 and SOCS3 stimulation and severity of disease in these models.

Keywords

AIDS-related cytomegalovirus retinitis; Murine cytomegalovirus; MAIDS; corticosteroids; suppressors of cytokine signaling (SOCS); SOCS1; SOCS3

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1. Introduction

Human cytomegalovirus (HCMV) is a β -herpesvirus capable of causing diseases of high morbidity and mortality in immunocompromised individuals. Among these diseases is AIDS-related HCMV retinitis. We have used for several years a mouse model of experimental murine cytomegalovirus (MCMV) retinitis in C57BL/6 mice with murine retrovirus-induced immunosuppression (MAIDS) to investigate the pathophysiology of AIDS-related HCMV retinitis (1). Whereas MCMV-infected eyes of healthy C57BL/6 mice without MAIDS are resistant to development of necrotizing retinitis (1), we have shown consistently in many studies that 80 to 100% of MCMV-infected eyes of C57BL/6 mice with MAIDS reproducibly develop severe necrotizing retinitis (1–4) that exhibits histopathologic features that mimic those observed in patients with AIDS-related HCMV retinitis (1).

We previously reported using the MAIDS model of experimental MCMV retinitis that suppressors of cytokine signaling (SOCS)1 and SOCS3 mRNAs are significantly stimulated to high amounts in MCMV-infected eyes (5), a finding that correlates with high frequency of retinitis. We also recently reported that SOCS1 and SOCS3 are significantly stimulated during MCMV infection of mouse macrophages (6). SOCS are a family of host proteins that are inducible negative regulators of signaling by Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways induced by antiviral type 1 interferons and other cytokines such as interleukin-6 (IL-6) (7). Although SOCS1 and SOCS3 have been implicated in several viral infections (reviewed in (8)), putative mechanisms involved in SOCS1 and/or SOCS3 stimulation during MCMV infection, and what role these proteins may play during MCMV infection or pathogenesis, remain unknown.

Because others have shown that development of experimental MCMV retinitis in eyes of C57BL/6 mice immunosuppressed by corticosteroid treatment results in reduced frequency of retinitis (~50%) (9) as well as significant macrophage loss (10), we sought to determine to what extent SOCS1 and SOCS3 mRNAs are stimulated in MCMV-infected eyes of drug-immunosuppressed mice. We report herein that SOCS1 and SOCS3 mRNAs and proteins are stimulated within MCMV-infected eyes in C57BL/6 mice during corticosteroid-induced immunosuppression, but at relatively low amounts. This outcome is correlated with less severe retinal pathologies and reduced ocular viral titers compared with our previous MAIDS studies (1–4). These data reveal a major difference between the two mouse models of MCMV retinitis and suggest further that MCMV-related stimulation of ocular SOCS1 and SOCS3 is directly correlated with the severity of MCMV retinitis.

2. Materials and methods

All animal work was performed in compliance with the National Institutes of Health guide for the care and use of laboratory animals. Retrovirus-induced immune suppression (MAIDS) was accomplished in adult female C57BL/6 mice following intraperitoneal infection with the murine retrovirus mixture LP-BM5 MuLV as described previously by us (1). Corticosteroid-induced immune suppression of adult female C57BL/6 mice was accomplished as described previously by others (9, 10) via intramuscular injection of the corticosteroid methylprednisolone acetate (2 mg/mouse, ~40 mg/kg) every 3 days, beginning

at day -2 relative to MCMV infection at day 0. The left eyes of the two groups of immune suppressed mice ($n = 5-8$ mice per group) were subjected to subretinal MCMV injection (1) with approximately 10^4 PFU of MCMV (Smith) contained within a 2- μ l volume of Dulbecco Modified Eagle's Medium (DMEM). The right eyes of all mice were injected subretinally with DMEM alone and served as controls. Left and right eyes from all mice were collected at 3, 6, or 10 days following subretinal MCMV injection and analyzed by real-time RT-PCR assay or ELISA for quantification of SOCS1 or SOCS3 mRNA or protein, respectively, using protocols previously described (4, 5). MCMV-infected eyes from parallel groups of MAIDS mice or corticosteroid-treated mice were collected at 10 days after subretinal MCMV infection and subjected to standard plaque assay for quantification of intraocular amounts of infectious virus (1) or processed for histopathology to determine the frequency and severity of MCMV retinitis using a scoring system described by us previously (1). For statistical analysis of data, MCMV-infected eyes were compared with their respective media-injected contralateral control eyes at the same time points by paired t test. P-values of <0.05 were considered statistically significant and were denoted in Figures where appropriate by asterisks as: * $p<0.05$ and ** $p<0.01$.

3. Results

After confirming in separate experiments our previously published findings (5) that SOCS1 (Figure 1A) and SOCS3 mRNA (Figure 1B) are significantly stimulated in MCMV-infected eyes of C57BL/6 mice with MAIDS at day 6 postinfection during retinitis development, we sought to investigate whether SOCS1 and SOCS3 mRNA are also stimulated in MCMV-infected eyes of C57BL/6 mice during corticosteroid-induced immunosuppression. We hypothesized that if these host proteins play a role in the pathogenesis of experimental MCMV retinitis, then they should be stimulated in more than one model of this disease. In sharp contrast to the robust stimulation of SOCS1 and SOCS3 mRNA exhibited during experimental MCMV retinitis of MAIDS mice (Figure 1A and 1B) (5), SOCS1 mRNA was only mildly, but not significantly, stimulated (Figure 1C) and SOCS3 mRNA was mildly, albeit significantly, stimulated (Figure 1D) in MCMV-infected eyes during corticosteroid-induced immunosuppression. At day 10 following subretinal injection, a trend toward increase in SOCS1 (Figure 1E) and SOCS3 (Figure 1F) protein production was also noted when MCMV-infected eyes of corticosteroid-induced immunosuppression were compared with contralateral uninfected control eyes by ELISA, although the observed increases were not statistically significant.

The low stimulation of MCMV-related ocular SOCS1 and SOCS3 mRNA expression during drug-induced immunosuppression, as compared with our findings during retrovirus-induced immunosuppression, compelled us to further explore this discrepancy between the two mouse models. We therefore sought to determine whether the failure to stimulate SOCS1 and SOCS3 correlated with decreased frequency and severity of experimental MCMV retinitis during corticosteroid-induced immunosuppression of C57BL/6 mice compared with a large body of data published previously by us for C57BL/6 mice with MAIDS at day 10 after subretinal MCMV inoculation with equivalent amounts of infectious virus (1-4). Only 40% (2/5) of MCMV-infected eyes of drug-immunosuppressed mice developed retinitis (Table 1), a frequency far less than that consistently observed by us for MCMV-infected eyes

of MAIDS mice (80 – 100%). The average severity score for these eyes using an established scoring scale of 1 – 4 (1) was also relatively mild (1.4; moderate atypical retinopathy) when compared with the range of severity scores previously published by us for MCMV-infected eyes of MAIDS mice (2 – 3.6; mild to moderate/severe necrotizing retinitis). As expected, amounts of intraocular virus was lower in MCMV-infected eyes of drug-immunosuppressed mice when compared with MCMV-infected eyes of MAIDS mice.

4. Discussion

Although MCMV highly stimulates SOCS1 and SOCS3 mRNA expression during experimental MCMV retinitis in retinitis-susceptible MAIDS mice, we were surprised to find that SOCS1 and SOCS3 mRNA and proteins were only mildly stimulated in another model of experimental MCMV retinitis of corticosteroid-induced immune suppression. Upon closer examination, however, we found a direct correlation between the amplitude of SOCS1 and SOCS3 stimulation and severity of retinal disease, further implicating these proteins in the pathogenesis of MCMV retinitis. As potent inhibitors of JAK/STAT, particularly of STAT1 and STAT3 (7), SOCS1 and SOCS3 could contribute to disease severity by dampening the signaling effects of antiviral interferons or other cytokines in a cell type-specific manner. Interferons or cytokines such as IL-6 which might otherwise suppress viral pathogenesis would therefore be rendered ineffective in cell types overexpressing SOCS1 or SOCS3.

These two models of experimental MCMV retinitis differ greatly in the timing of immune suppression, the mechanisms by which immune cells are rendered defective, and the availability of immune cell populations during development of retinitis. One major difference between these mouse models is macrophage number. MAIDS is associated with increased macrophage numbers over a period of weeks and at the time when animals are susceptible to MCMV retinitis (11). By contrast, corticosteroids such as methylprednisolone poison nearly all aspects of the innate and adaptive immune system within days, including macrophages (12). Therefore, whereas mice with MAIDS experience an increase in macrophage number, corticosteroid-induced immune suppression very quickly results in significant loss of macrophage numbers. These two methods of distinct immune suppression therefore uniquely affect immune cell populations, particularly macrophage populations and cytokine responses to infection. It is intriguing to speculate that this outcome may be due, in part or in particular, to quantitative differences in macrophage populations observed in the two mouse models of experimental MCMV retinitis, especially because MCMV stimulates SOCS1 and SOCS3 in mouse macrophages grown in culture (6).

The MAIDS model of MCMV retinitis by us (1) uses MCMV (Smith) whereas the corticosteroid model of MCMV retinitis by others (9) uses MCMV (K151). It is unlikely, however, that strain differences would account for the disparate SOCS1 and SOCS3 findings. Both are laboratory virus strains, and both are passaged through salivary glands prior to intraocular injection. Moreover, we directly compared herein both mouse models using MCMV (Smith) and observed the same frequency of retinitis reported previously for MCMV (K151) (9).

One might argue that methylprednisolone acetate as a glucocorticoid will influence SOCS1 and/or SOCS3 expression within the eyes of mice immunosuppressed with this corticosteroid even without intraocular MCMV infection. In fact, evidence is emerging that there is indeed cross-talk between glucocorticoids and SOCS1 and SOCS3. One study (13) showed that SOCS1 and type 1 interferons are glucocorticoid targets for regulating STAT1 activity in macrophages of C57BL/6 mice through induction of SOCS1, thereby contributing to overall inflammation suppression effectiveness. Another study (14) showed that glucocorticoids in cultures of mouse hepatocytes acted on IL-6-induced gene expression through reduction of SOCS3. Whether such mechanisms operate within MCMV-infected eyes of methylprednisolone acetate-treated mice in our study remains to be determined. Differences in level and mode of virus entry into ocular cells (15) influenced by glucocorticoid versus retrovirus-induced immunosuppression is another possibility to be explored.

In summary, herein we demonstrate that the MCMV-infected eyes of corticosteroid-immunosuppressed mice compared with MCMV-infected eyes of mice with MAIDS revealed sharply different patterns of SOCS1 and SOCS3 mRNA production. This was concurrently observed with a reduction in frequency and severity of retinitis and ocular titers in the corticosteroid-induced immunosuppression model compared with MAIDS-related MCMV retinitis (1–4), suggesting that SOCS1 and SOCS3 expression may be involved in the severity of this disease. These data provide further evidence that SOCS1 and SOCS3 are involved in the pathogenesis of experimental MCMV retinitis during retrovirus-induced immunosuppression, and it lays crucial groundwork that may, in the future, lead to new insights or therapeutic targets for the clinical disease.

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Highlights

- Mice with MAIDS show significant increase of SOCS1 and SOCS3 in MCMV-infected eyes.
- Mice treated with corticosteroid show reduced SOCS1 and SOCS3 in MCMV-infected eyes.
- Mice treated with corticosteroid show reduced frequency of MCMV retinitis.
- Reduced frequency of MCMV retinitis correlates with low levels of SOCS1 and SOCS3.

Figure 1. SOCS1 and SOCS3 expression is different in two different models of experimental MCMV retinitis

Groups of adult C57BL/6 mice with MAIDS of 10 weeks' duration (**A, B**) and groups of adult C57BL/6 mice immune suppressed by corticosteroid treatment (**C–F**) were subretinally injected with 10^4 PFU of MCMV (left eyes) or DMEM (right eyes) as described in Materials and Methods. Whole eyes were harvested at indicated times following subretinal MCMV injection and assessed by real-time RT-PCR assay using the comparative 2^{-C_t} method for quantification of SOCS1 mRNA expression within MCMV-infected eyes of MAIDS mice (**A**), SOCS3 mRNA expression within MCMV-infected eyes of MAIDS mice (**B**), SOCS1 mRNA expression with MCMV-infected eyes of corticosteroid-immunosuppressed mice (**C**), and SOCS3 mRNA expression of MCMV-infected eyes of corticosteroid-immunosuppressed mice (**D**). Ocular SOCS1 protein (**E**) and SOCS3 protein (**F**) were quantified at day 10 by ELISA from mice with corticosteroid-induced immunosuppression. Means $\pm$ SD of $n = 5$ –8 mice per group are shown. * $p < 0.05$ and ** $p < 0.01$ for MCMV-infected eyes compared with media-injected controls.

Table 1

Frequency and Severity of Retinitis and Ocular Viral Load in Two Different Models of Experimental MCMV Retinitis

Method of Immune Suppression of C57BL/6 mice	Frequency (%) of Full-Thickness Retinal Necrosis	Retinitis Severity Score (% of max. possible score) ^c	Ocular MCMV Titer (PFU/eye)
Retrovirus (MAIDS)^a	80–100%	2–3.6 (50–90%)	1–5×10 ⁴
Corticosteroids^b	40%	1.4 (35%)	5.3×10 ³

All data are from whole eyes collected at day 10 following subretinal injection of ~10⁴ PFU/eye of MCMV (Smith)

^a: Data compiled from published MAIDS studies in C57BL/6 mice (1–4)

^b: Data from present study in C57BL/6 mice with corticosteroid-induced immunosuppression

^c: Scoring scale of 0 – 4 as described by us previously (1)