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Original article

Homeostatic expansion of CD4$^+$ T cells upregulates VLA-4 and exacerbates HSV-induced corneal immunopathology

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Abstract

Using a viral-induced immunopathology model, we showed that when CD4$^+$ T cells were allowed to undergo homeostatic expansion prior to ocular herpes simplex virus infection, mice developed more severe inflammatory lesions with the increased severity associated with enhanced effector function of ocular CD4$^+$ T cells, and blocking their functional activity reduced the lesion severity. Additionally, homeostatically expanded CD4$^+$ T cells upregulated VLA-4, and in vivo administration of anti-VLA-4 mAb significantly decreased the homeostatic proliferation. Furthermore, blocking of VLA-4 interaction also diminished the infiltration of CD4$^+$ T cells into the cornea and decreased lesion severity. Our results imply that homeostatic expansion of T cells, as could occur in a virus-induced lymphopenia, may generate cells with enhanced effector function that can contribute to tissue damage.
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1. Introduction

In healthy animals, the peripheral lymphocyte pool size remains quite constant. Conditions of lymphopenia usually induce lymphocytes to proliferate so as to restore the pool size [1]. This event, refereed to as homeostatic proliferation, involves naïve and memory lymphocytes and various factors such as self peptide reactivity [2,3], clonal competition [4,5] and different cytokines regulate their cell division [6–8]. These homeostatically dividing cells express many activation markers characteristic of memory cells and are functionally active [9,10]. Recently, it has been argued that lymphopenia may result in the homeostatic expansion of autoreactive T cells that mediate some autoimmune diseases [11,12]. In addition, T-cell homeostatic expansion (HE) may also result in the enhanced anti-tumor activity of CD8$^+$ T cells [13]. Several viral infections induce a temporary or long term lymphopenia [14–19]. It is not clear what role, if any, the vigorous compensatory lymphocyte expansion that may follow plays in anti-viral immunity and pathology. Certain reports claim that these events may explain the association of some viruses with autoimmunity [12]. However, autoimmune disease expression is a very rare event after any human virus infection and no single human virus, whether causing lymphopenia or not, has regularly been associated with human autoimmunity [20]. A common event, however, during recovery from many virus infections is collateral tissue damage caused by activated T cells [20]. In some locations, such as the brain, lung or the eye, this can result in significant morbidity. It is not clear if the T cells involved in the tissue damage represent viral antigen specific, homeostatically expanded autoantigen specific, or nonspecific cells that react with neither viral nor autoantigens.

Herpes simplex virus -1 (HSV-1) infection of the mouse cornea can lead to the development of an immunopathological

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lesion, termed herpetic stromal keratitis (HSK). The pathogenesis of HSK, as studied in the mouse, involves numerous components, but $CD4^+$ T cells appear to be the principal orchestrators of the immunopathology [21]. One of the possible ways by which $CD4^+$ T cells can get activated in the eye involves bystander activation of T cells after ocular HSV-1 infection [22]. Once activated, $CD4^+$ T cells secrete Th1 specific cytokines that play an important role in orchestrating immunoinflammatory reaction in the cornea [23]. In the present communication, we determined that homeostatically expanded $CD4^+$ T cells cause more tissue damage to virus-infected cornea when compared to infected control animals. Our results demonstrated that homeostatic expansion of $CD4^+$ T cells enhanced their effector functions that lead to increased corneal lesion severity. Interestingly, homeostatically expanded transgenic T cells, not demonstrably reactive with either viral or autoantigens, significantly contributed to lesion expression but they were less pathogenic when unable to secrete IFN-γ. Additionally, cells undergoing homeostatic proliferation upregulated VLA-4, and in adoptive transfer experiments blocking VLA-4 with specific antisera significantly decreased the ocular infiltration of $CD4^+$ T cells and the consequent severity of ocular lesions. Our results support the idea that tissue damage evident during recovery from viral infections may be the consequence of homeostatically expanded cells which in the stromal keratitis (SK) model can be mediated by cells of non-viral antigen specificity. Whether such a mechanism occurs as a result of homeostatic expansion in natural infection models that cause lymphopenia requires further evaluation.

2. Materials and methods

2.1. Mice and virus

Female 6- to 8-week-old BALB/c SCID (H-2^d), mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and DO11.10 (OVA-TCR Tg mice), DO11.10 IFN-γ −/− mice were housed and bred in the animal facilities at the University of Tennessee. The DO11.10 mice were kindly provided by C. Weaver (University of Alabama, Birmingham, AL). Approximately 70% of $CD4^+$ T cells in the DO11.10 mice were KJ1.26.1 (anti-OVA-TCR Ab) positive. SCIDS were kept in our special pathogen free facility. All experimental procedures were in complete agreement with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. HSV-1 RE was propagated and titrated on Vero cells (ATCC CCL81) using standard protocols. The virus was stored in aliquots at −80 °C until use.

2.2. T-cell purification, adoptive transfer and virus infection

DO11.10 $CD4^+$ T cells were purified by positive selection with CD4 microbeads as per manufacturer's protocol (Miltenyi Biotec, Auburn, CA). The purified $CD4^+$ T cells were incubated with anti-CD25 mAb (10 μg/10^8 cells) at 4 °C for 30 min. This was followed by the addition of baby rabbit complement (BRC) into the cell culture at 1:200 dilution and cells were kept at 37 °C for 45 min (Cedarlane Laboratories). Next, $CD4^+CD25^-$ T cells obtained after BRC killing were labeled with 2.5 μm CFSE (Molecular Probes, Eugene, OR) as reported by others [24] and suspended in PBS for injection. Each mouse received 10^7 CFSE-labeled $CD4^+$ T cells via lateral tail vein injection. In test group of animals, $CD4^+$ T cells underwent homeostatic expansion for 7 days prior to ocular HSV infection while control group of animals received $CD4^+$ T cells 24 h before ocular infection. To enumerate the infiltration of $CD4^+$ T cells into the diseased corneas, mice from both test and control groups were terminated at different time-points post infection and liberase digested corneal samples were stained for $CD4^+$ T cells by flow cytometry. When indicated, some mice received intraperitoneal ammonium sulfate cut anti-VLA-4 mAb (1 mg/mouse) post adoptive transfer. In certain experiments, role of OVA-specific T cells in determining the corneal lesion severity after homeostatic expansion was ascertained in SCID recipients. Since approximately 70% of $CD4^+$ T cells in the DO11.10 mice are KJ1.26.1 (anti-OVA-TCR Ab) positive, $CD4^+KJ^+$ T cells purified from DO11.10 IFN-γ−/− mice were mixed with $CD4^+KJ^-$ T cells from DO11.10 mice in 70: 30 ratio (7×10^6:3×10^6) and adoptively transferred into BALB/c SCID. SCID mice were ocularly infected with HSV-1 RE at a dose of 10^4 PFU/eye 7 days post adoptive transfer and clinical scoring of the eyes was done as described earlier [22].

2.3. Flow cytometric analysis

2.3.1. Cell preparation

Single-cell suspensions were prepared from corneas, draining lymph nodes (cervical and submandibular) and spleen of recipient mice at different time points post-infection. Corneas were digested with Liberase (Roche Diagnostics). Briefly, a small incision was made at the junction of the limbus and the corneal cap was carefully removed. The pooled corneas were incubated with 60 U/ml liberase for 60 min at 37 °C in a humidified atmosphere of 5% CO_2. After incubation, the corneas were disrupted by grinding with a syringe plunger on a stainless steel mesh and a single-cell suspension was made in complete RPMI 1640 medium. Cells were counted with trypan blue exclusion with high viability.

2.3.2. Staining for flow cytometry

The single-cell suspension obtained from draining lymph nodes (DLN) and corneal samples were stained for cell surface CD4 molecules by FACS. Briefly, a total of 1×10^6 cells were first blocked with an unconjugated anti-CD32/CD16 mAb for 30 min. in FACS buffer followed by addition of FITC-labeled anti-CD4 mAb for an additional 45 min. For intracellular staining, surface-labeled cells were permeabilized with permeabilization buffer (BD Biosciences, Mountain View, CA) and PE-labeled anti-IFN-γ mAb was added. Finally, the cells were washed three times and samples were acquired on a FACScan (BD Biosciences). The data were analyzed using CellQuest 3.1 software (BD Biosciences).

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