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Original article

Interleukin-17 enhanced immunoinflammatory lesions in a mouse model of recurrent herpetic keratitis

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Abstract

Interleukin-17 (IL-17), mainly produced by activated (memory) T cells, has been found in the corneas from herpetic stromal keratitis (HSK) patients. To better understand the role of IL-17 and to optimize fidelity to human recurrent HSK, in this study, we utilized a mouse model of recurrent HSK, examined the expression of IL-17 and Th17 cells, and determine the alterability of virus-induced corneal inflammation after anti-IL-17 antibody treatment during murine recurrent HSK. We found that Th17 cells were obviously up-regulated in both cornea and DLNs of recurrent mice. Peak IL-17 protein present in recurrent cornea in conjunction with peak opacity mediated by $CD4^+$ T cells. Systemic administration of anti-IL-17 antibody resulted in a diminished severity of corneal opacity, neovascularization, and $CD4^+$ T cells infiltration compared to control. Anti-IL-17 treatment down-regulated the mRNA and protein levels of TNF-α expression in recurrent corneas, and decreased HSV-specific DTH responses. Our results indicate that elevated IL-17 expression may be involved in the development of recurrent HSK. The likely mechanisms of action for IL-17 are through up-regulating TNF-α expression and promoting HSV-specific DTH responses. Thus, IL-17 might constitute a useful target for therapeutic intervention in recurrent HSK.

Keywords: Interleukin-17; Herpes simplex virus; Herpetic stromal keratitis; Delayed type hypersensitivity; Recurrent mouse model

1. Introduction

Herpetic stromal keratitis (HSK) is an immune-mediated disease of the corneal stroma that results from an infection by herpes simplex virus type 1 (HSV-1) [1,2]. HSK in humans most often occurs long after primary infection abates, when reactivated virus returns to the eye from latency sites in neuronal tissues. Repeated corneal inflammations induce corneal opacities due to cellular infiltration and scarring, which frequently leads to severe visual impairment. Traditional antiviral therapy is limited because the pathogenesis of recurrent HSK involves mainly immune-mediated mechanisms rather than viral cytopathic effects [3]. Immune system plays a key role in the development of these pathologic changes in recurrent HSK [4,5].

Murine models have provided most of our current understanding of the immunologic involvement in HSK [1]. The $CD4^+$ T lymphocyte subset, which possesses helper function, is the principal mediator for HSK [1]. Initial experimental studies, using the mouse model of primary infection, have shown that the $CD4^+$ T cells, predominantly of the T helper 1 (Th1) type, are major contributors to the development of HSK [6–8]. There is an evidence that the Th1-type cytokines interferon-γ (IFN-γ) and interleukin (IL)-2 are secreted during the progression of disease, and neutralization of these cytokines significantly reduces the severity of HSK. Th2 cells that produce typically IL-4 and IL-10 are a possible regulator that controls the severity of HSK. For instance, IL-10 and IL-4 appears to be expressed during late stages of HSK concordant with diminishing inflammation [9]. Moreover, HSK is alleviated by topical administration of recombinant IL-10 [10].

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But subsequent work, using the recurrent model of HSK, have shown that recurrent HSK may not be characterized by a classic Th1 or Th2 response, both Th1 and Th2 cytokines present in the cornea during recurrent HSK [11,12] and IFN-γis not required for recurrent HSK [13].

Recently, a third subset of effecter helper T cells that exhibit functions distinct from Th1 and Th2 cells and preferentially produce interleukin-17 (IL-17) (named Th17 cells) has been discovered [14–16]. Accumulating data suggest that Th17 cells mediate immune responses, promote chronic inflammation and autoimmunity [17,18]. Furthermore, Th17 cells also appear to play a role in protection against extracellular bacterial or fungal infections [18,19]. Th17 cells are responsible for host defense by producing pro-inflammatory cytokines most notably IL-17 [20]. The IL-17 family consists of six members including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17, also known as IL-17A, is the prototype member of the IL-17 family.

To date, Th17 cells have been associated with HIV infection and respiratory syncytial virus infections [21,22]. The functionality of IL-17 has also attracted much attention during HSV-1 infection. Recent work by Maertzdorf showed that IL-17 is expressed in corneas of patients with HSK, and that the IL-17 receptor is constitutively expressed by cultured human corneal stromal fibroblasts [23]. In addition, experimental studies, using the ocular primary infection mouse model of HSK, have shown that IL-17 is elevated in the murine cornea after HSV-1 infection, and contributes to virus-induced corneal inflammation [24,25]. However, what role of IL-17 in recurrent HSK is unclear, which needs to be further researched.

In the present work, we tested the expression of IL-17 and Th17 cells in recurrent HSK mice, and examined the possible therapeutic benefits of anti-IL-17 antibody treatment. We hypothesized that IL-17 exerted effects of pro-inflammatory on recurrent HSK by augmenting tumor necrosis factor (TNF)-α expression, the major cytokine that contributes to delayed-type hypersensitivity (DTH) responses. This study may facilitate identification of novel molecular targets for HSK therapy.

2. Materials and methods

2.1. Mice

Female BALB/c mice, 6–7 weeks old, weighing about 20 g each, were purchased from the animal center of Beijing University (Beijing, China). All experimental manipulations were undertaken in accordance with the institutional guidelines for the care and use of laboratory animals.

2.2. Virus and primary infection

The virus used in these studies was the human isolate HSV-1 McKrae strain. A plaque-purified stock was grown and assayed on VERO cells in Dulbecco's modified Eagle's medium (DMEM), containing 5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37 °C in a humidified incubator containing 5% CO_2.

The BALB/c mice were inoculated by the corneal route with HSV-1 McKrae strain as follows. Briefly, after the mouse was intraperitoneally anesthetized with 0.5% pentobarbital (45 mg/kg bodyweight), the corneal surface of the right eye was incised in a cross shaped pattern with a sterile with a 27-gauge needle, and a 5 μl drop containing 1×10^5 plaque-forming units (PFU) of HSV-1 McKrae was applied to the eye and gently massaged with the eyelids. Each mouse received an intraperitoneal injection of 1 ml of pooled human serum (anti-HSV reactivity with an effective dose for 50% viral neutralization of 1:800) concurrent with infection. Administration of anti-HSV antibodies at the time of ocular infection has been demonstrated to have a protective effect during primary infection, resulting in mice with clear corneas and the ability to undergo recurrent virus shedding and herpetic ocular disease following UV-B irradiation [11,13,26].

To detect ocular virus shedding after corneal HSV-1 inoculation, material from eye swabs was plated onto VERO cells, which were then monitored for cytopathic effects 48 and 96 h later. A successful corneal HSV-1 inoculation was defined as virus shedding in tear film on any day from day 1–7 post inoculation.

2.3. UV-B irradiation and virus reactivation

Six weeks after inoculation, reactivation of latent HSV-1 infection was induced by Ultraviolet B (UV-B) irradiation [11,13]. The eyes of all mice were examined for corneal opacity before irradiation, and only animals with clear corneas were used. In brief, the right eyes of every anesthetized latently-infected mice and control mock-infected mice were exposed to 250 mJ/cm^2 of UV-B light using a TM20 Chromato-Vu transilluminator (UVP, Inc., San Gabriel, CA), which emits UV-B at a peak wavelength of 302 nm. The eye swab materials from the mice were obtained before (day 0) and on days 1–7 post UV-B irradiation and were cultured in VERO cells as described above to detect recurrent virus shedding from the cornea. Recurrent disease was defined as stromal opacification for more than two consecutive days and virus shedding in tears on any day from day 1 to day 7 post UV-B irradiation (day 0 swabs served as control).

2.4. Isolation of infiltrating cells in the corneal stroma and draining lymph nodes (DLNs) and flow cytometry analysis of IL-17 expression on T lymphocytes

The corneas and cervical DLNs from recurrent HSK mice, UV-B control mice and normal mice were aseptically removed on day 7 after UV-B irradiation. For flow cytometry measurement of the infiltrating cells of the cornea, six corneas per group were collected by dissecting the corneal buttons above the limbus with a scalpel. The pooled corneas were incubated with 60 U/ml Liberase (Roche Diagnostics Inc., Indianapolis, IN) for 60 min at 37 °C in a humidified