Cell Biology International 33 (2009) 534–541

Cell Biology International

www.elsevier.com/locate/cellbi

Treatment with FTY720 during the induction or effector phase suppresses the development of experimental allergic conjunctivitis in mice

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Received 24 October 2007; revised 1 March 2008; accepted 10 February 2009

Abstract

Purpose: To investigate whether experimental allergic conjunctivitis (EC) can be suppressed by treatment with the immunomodulatory drug FTY720, which reduces the recruitment of effector T cells into inflammatory sites.
Methods: BALB/c mice were actively immunized with ragweed (RW) and then injected intraperitoneally with FTY720 on days 0, 2, 4, 6 and 8 (induction phase treatment) followed by challenge on day 10 with RW-containing eye drops. Alternatively, naïve mice that received RW-primed splenocytes were injected intraperitoneally with FTY720 on days 2 and 4 (effector phase treatment) followed by RW challenge on day 4. Twenty-four hours after RW challenge, conjunctivas and spleens were harvested for histology or immunohistochemistry, and flow cytometric analysis or cytokine assays, respectively.
Results: FTY720 treatment during the induction phase suppressed the conjunctival infiltration of T cells as well as eosinophils and macrophages. The splenocytes from induction phase-treated mice contained significantly less $CD4^+$ and $CD8^+$ T cells and showed significant suppression of Th2 but not Th1 cytokine production. Effector phase treatment with FTY720 suppressed conjunctival eosinophil infiltration.
Conclusions: These data demonstrate that FTY720 treatment during the induction phase decreases the absolute number of $CD4^+$ and $CD8^+$ T cells in the spleen and suppresses Th2 cytokine production by splenocytes. This leads to the suppression of EC. FTY720 treatment also suppresses EC when delivered during the effector phase. Thus, FTY720 treatment may be suitable for treating severe forms of vernal keratoconjunctivitis.

Keywords: Allergic conjunctivitis; Eosinophil; FTY720; Splenocyte; Th2

1. Introduction

Massive infiltration of inflammatory cells such as eosinophils into the conjunctiva is a hallmark of severe forms of allergic conjunctivitis (AC) such as vernal keratoconjunctivitis (VKC) (Friedlaender et al., 1984). Although AC is mediated by interaction between antigen (Ag), IgE, and mast cells (Graziano et al., 2001), we have demonstrated that IgE-mediated mast cell activation on its own cannot induce massive conjunctival infiltration of eosinophils in mice developing experimental allergic conjunctivitis (EC) (Fukushima et al., 2005a). However, this eosinophilic infiltration is observed when Ag-primed T cells are transferred and the animals are challenged with the priming Ag (Fukushima et al., 2003, 2006a). Thus, Ag-specific T cells appear to play an essential role in the infiltration of inflammatory cells into the conjunctiva. Supporting this is that immunohistochemical analyses of VKC patient conjunctivas have revealed the presence of large number of T cells as well as eosinophils (Bonini et al., 1995).

FTY720 is a chemical derivative of a metabolite of the ascomycete *Isaria sinclairii* that has been shown to effectively

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doi:10.1016/j.cellbi.2009.02.002

suppress Th1-mediated graft rejection in animals (Suzuki et al., 1996; Furukawa et al., 2000) and humans (Budde et al., 2003; Tedesco-Silva et al., 2006). Unlike the conventional immunosuppressants cyclosporine A and FK506, FTY720 does not impair the activation, expansion and memory function of T cells. Instead, it exerts its immunomodulatory effects by reducing the recirculation of effector T cells and their recruitment to peripheral lesions (Chiba, 2005). FTY720 has been shown to exert suppressive effects on autoimmune diseases such as experimental autoimmune uveoretinitis (EAU) (Kurose et al., 2000), which is also mediated by Th1 cells. More recently, it has been shown to significantly reduce eosinophil infiltration into the lung in experimental airway inflammation induced by the transfer of Ag-specific Th2 cells (Sawicka et al., 2003). However, it remains unclear whether FTY720 can suppress EC, which is also a Th2 cell-mediated disease, especially since we have found mechanistic differences between experimental airway inflammation and EC. For instance, while the interaction between inducible co-stimulator (ICOS) and its ligand B7-related protein-1 (B7RP-1) is important for the development of airway inflammation during the effector phase (Gonzalo et al., 2001), it is not essential for the development of EC (Fukushima et al., 2005b). Here, we examined whether FTY720 treatment can suppress the development of EC.

2. Materials and methods

2.1. Mice

Inbred wild-type (WT) Balb/c mice were purchased from Japan SLC Inc., Hamamatsu, Shizuoka, Japan. The mice were kept in pathogen-free conditions at the animal facility of Kochi Medical School and age- and gender-matched mice were used when they were 6- to 12-weeks-old. All research adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Reagents

Short ragweed pollen (RW) was purchased from Polysciences, Inc, Warrington, PA. RW extract was obtained from LSL Co. Ltd, Tokyo, Japan. Aluminum hydroxide (alum) was purchased from Sigma, St. Louis, MO. FTY720 was obtained from Novartis Pharma AG (Basel, Switzerland). The following antibodies (Abs) were purchased as follows: FITC-labeled anti-CD3 (145-2C11), FITC-labeled anti-CD4 (GK1.5), biotin-labeled anti-CD8 (53−6.7) and biotin-labeled anti-mouse CD45R/B220 (RA3-6B2) were from eBioscience, San Diego, CA, FITC-labeled anti-CD45R/B220 (RA3-6B2) and streptavidin-PE were from BD Biosciences, Franklin Lakes, NJ, biotin-labeled anti-F4/80 (A3-1) and biotin-labeled rat anti-mouse CD3 (CT-CD3) were from Caltag Laboratories, Burlingame, CA, and biotin-labeled anti-mouse CD11b (M1/70.15) was obtained from Invitrogen, Carlsbad, CA. Rabbit anti-mouse major basic protein (MBP) was kindly provided by Dr. James J. Lee (Mayo Clinic, Scottsdale, AZ). The Bio-Plex Cytokine Assay Panel (Bio-Rad, Hercules, CA) was used for cytokine analysis.

2.3. EC induction by active immunization and treatment with FTY720 during the induction phase

The left hind-footpad and tail base were each injected with 50 μl of RW adsorbed on alum (50 μg RW and 675 μg alum). The mice were injected intraperitoneally with 2 or 20 μg of FTY720 ($n = 10$ per group) or the same volume of distilled water (DW, $n = 10$) on days 0, 2, 4, 6 and 8 after immunization (10 or 100 μg of FTY720 in total). Ten days later, the eyes of the immunized mice were challenged with RW in PBS (2 mg in 10 μl per eye). Twenty-four hours later, the eyes and spleens were harvested for histological and immunohistochemical analysis, and flow cytometric analysis and cytokine assays, respectively. In a separate experiment ($n = 10$ per group), the conjunctivas and spleens were harvested 24, 72 and 168 h after RW challenge to evaluate conjunctival eosinophil infiltration and perform flow cytometric analysis, respectively.

2.4. EC induction by adoptive transfer of in vitro-stimulated RW-primed splenocytes and treatment with FTY720 during the effector phase

Naïve Balb/c mice were immunized with RW in both their left hind-footpad and tail base as described above. Ten days later, harvested splenocytes at a concentration of 10^7 cells per ml were cultured with RW extract at final concentrations of 5 μg/ml in 75-cm^2 flasks in a final volume of 20 ml RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, ICN Biomedical Japan Co., Tokyo, Japan), 2-mercaptoethanol (2-ME, 5×10^{-5} M), L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). After incubation for 72 h at 37 °C in a humidified atmosphere with 5% CO_2, 2×10^7 splenocytes were injected intraperitoneally into syngeneic naïve Balb/c mice. After the splenocyte transfer, the mice were injected intraperitoneally on days 2 and 4 with 2 or 20 μg of FTY720 (4 or 40 μg of FTY720 in total, $n = 10$ per group). As a control, the same volume of DW was injected ($n = 10$ per group). Two hours after the second FTY720 injection, the eyes of the recipient mice were challenged with RW in PBS (2 mg in 10 μl per eye). Twenty-four hours later, the eyes were harvested for histological analysis.

2.5. Histological analysis

The eyes including the conjunctivas were harvested and fixed in 10% buffered formalin. Vertical 2 μm-thick sections were cut and stained with Giemsa. Infiltrating eosinophils in the lamina propria mucosae of the tarsal and bulbar conjunctivas throughout each section were counted by two blinded observers. The sections counted were those of the central portion of the eye, which included the pupil and optic nerve