The role of regulatory T cells in mice with experimental autoimmune uveitis – a preliminary study

YUKI NANKE[1], NOBUYUKI ISHIGURO[2], TORU YAGO[1], TSUYOSHI KOBAHIGAWA[1], TOKUYASU HASHIDA[2], SHIGERU KOTAKE[1]

[1]Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo, Japan
[2]Department of Ophthalmology, Osaka University, Osaka, Japan

Abstract

The current study investigates the role of regulatory T (Treg) cells in the pathogenesis of experimental autoimmune uveoretinitis (EAU), a model for human uveitis, induced by the retinal antigen interphotoreceptor retinoid-binding protein (IRBP).

C57BL/6 mice were immunized with human IRBP-20 ($IRBP_{1-20}$). CD4+CD25+^{bright} T cells (Treg cells) from the peripheral blood of mice were measured using two color flow cytometry. The severity of EAU was evaluated histopathology.

Inflammation was seen on days 17 and 21. The number of Treg cells in CD4+ T cells was elevated on day 9 and decreased on day 21.

The number of Treg cells in the peripheral blood was elevated on day 9 when inflammation was not noted. Treg cells may play a pivotal role in the pathogenesis of EAU induced by IRBP.

Key words: *uveitis, ocular attack, Behçet's disease, regulatory T, EAU.*

(Centr Eur J Immunol 2012; 37 (2): 102-105)

Introduction

Experimental autoimmune uveoretinitis (EAU) serves as a model of human endogenous uveitis. Behçet's disease (BD) is polysymptomatic, with recurrent oral and genital ulceration, and uveitis with a chronic course and an unknown cause [1]. The pathology of the lesions consists of widespread vasculitis. The eyes [2], skin, joints [3], oral cavity, blood vessels, and central nervous system are usually involved. Behçet's disease patients sometimes become blind because of uveitis. CD4+CD25+^{bright} T cells are a population of regulatory T (Treg) cells responsible for the active suppression of autoimmunity [4]. Treg cells play an important role in the pathogenesis of autoimmune disorders. These autoimmune disorders can be prevented by the infusion of Treg cells [4]. Treg cells also inhibit previously activated CD4+CD25(–) autoreactive T-cell clones [4]. These findings indicate that these cells may contribute to regulation of the immune response in humans. We reported that Treg cell levels in peripheral blood before ocular attack were significantly lower than the normal level in a prospective study [5]. In addition, a decreased percentage of Treg cells in peripheral blood may be a predictive marker of ocular attack in BD patients. In the current study, we investigated the role of Treg cells in the pathogenesis of EAU, a model of human uveitis, induced by the retinal antigen interphotoreceptor retinoid-binding protein (IRBP).

Material and methods

C57BL/6 mice were immunized with human $IRBP_{1-20}$ (IRBP1-20, GPTHLFQPSLVLDMAKVLLD). Interphotoreceptor retinoid-binding protein was synthesized by Sigma Genousys Japan (Isikari City, Hokkaido, Japan) emulsified in CFA ($n = 12$, control mice: $n = 9$). On day 20, 90% of mice developed uveitis. The eyes were enucleated on days 7, 14, or 21 after immunization. The severity of EAU

Correspondence: Dr. Yuki Nanke, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan, phone +81-3-5269-1725, fax +81-3-5269-1726, e-mail: ynn@ior.twmu.ac.jp

was evaluated by histopathology. The histological severity was graded on a scale of 0-4, as follows: grade 0, absence of apparent inflammation; grade 1, mild protein exudation in the aqueous humor and vitritis; grade 2, iris synechiae; grade 3, fibrin clumps in the anterior chamber; and grade 4, opaque anterior chamber [6]. The histology score was based on the degree of cell infiltration, vasculitis, granuloma formation, photoreceptor cell damage in the retina and choroid and retinal detachment in the eye. Experimental autoimmune uveoretinitis severity was double-blindscored by two ophtalmologists. Treg cells from the peripheral blood of mice were measured employing two color flow cytometry. Dual color flow cytometry was performed to quantify all populations of Treg cells. Cells were stained with anti-CD4-FITC (Beckman Coulter Inc., Florida, USA) and anti-CD25-PE (Beckman Dickinson Immunocytometry System, California, USA). Flow cytometry was performed using a Beckman Dickinson FACScan instrument (Becton Dickinson, Franklin Lakes, New Jersey, USA). The proportion of total CD4+CD25+ T cells was determined by the level of isotype control fluorescence. The CD4+CD25+^{bright} fraction was determined by more than a 10^2 fluorescence intensity, with the same settings used in all patients and controls (Fig. 1). Lymphocytes were gated according to forward and size scatter, and ten thousand cells were analyzed with CellQuest software (Becton Dickson). Results were compared using arbitrary units (AU) of the mean fluorescence intensity (MFI) as a relative measure of CD25 expression.

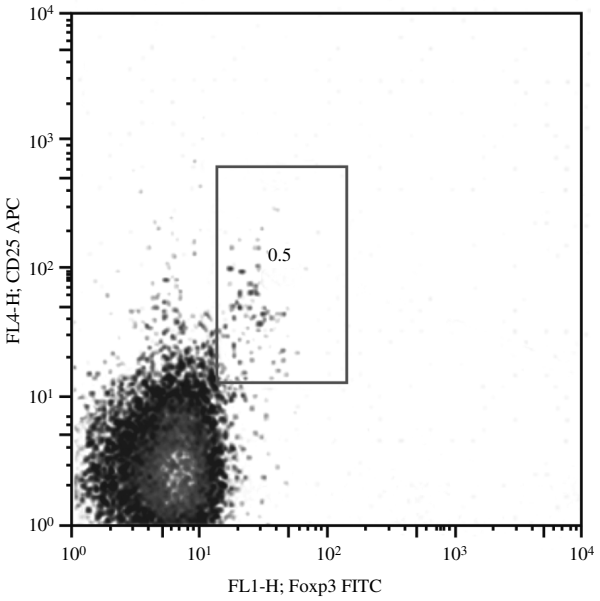

Fig. 1. Lymphocytes were gated according to forward and side scatter (FSC/SSC) from peripheral blood mononuclear cells, and then screened by flow cytometry for the presence of CD4+CD25+T and CD4+CD25+^{bright} T cells. Representative FACS staining from one normal control subject is shown

Statistical analysis

Significance was analyzed using the Mann-Whitney test and Wilcoxon's rank sum test. The results are expressed as the means ±SD, and considered significant at a p-value of < 0.05.

Results

No inflammation was seen on day 4 ($n = 21$). On day 17, mild detachment was observed in the vitreous chamber and the thickening grade was 2. In the retina, cell infiltration and vasculitis were seen, with the destruction of the retinal structure being grade 3. On day 21, severe detachment was observed in vitreous, and the thickening grade was 3. In the retina, cell infiltration and vasculitis were seen, and the destruction of the retinal structure was grade 3 (Fig. 2). Figure 3 shows the ratio of Treg cells. The number of Treg cells was elevated on day 9 and decreased on day 21. We did not measure the number of Treg cells on day 17.

Discussion

Uveitis is a sight-threatening disease affecting the neural retina and uvea. The most common and classical murine model is EAU [6]. In this study, we demonstrated inflammation in EAU mice on day 17 and day 21. The number of Treg cells among CD4+ T cells in the peripheral blood was elevated on day 9 when inflammation was not seen, and was decreased on day 21. Thus, in mice, the number of Treg cells was elevated before the development of uveitis, in contrast to human BD [5].

We have reported that, in humans, the percentages of Treg cells among CD4+T cells from BD are significantly decreased before compared with those after ocular attack [5]. In addition, more importantly, these levels before ocu-

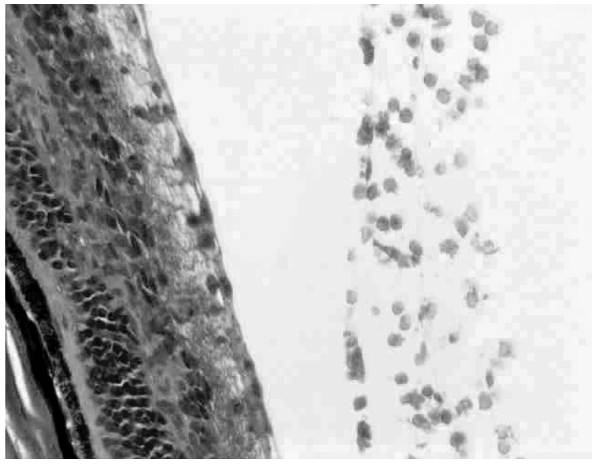

Fig. 2. The severity of experimental autoimmune uveoretinitis was evaluated by histopathology. HE staining, original magnification 40×

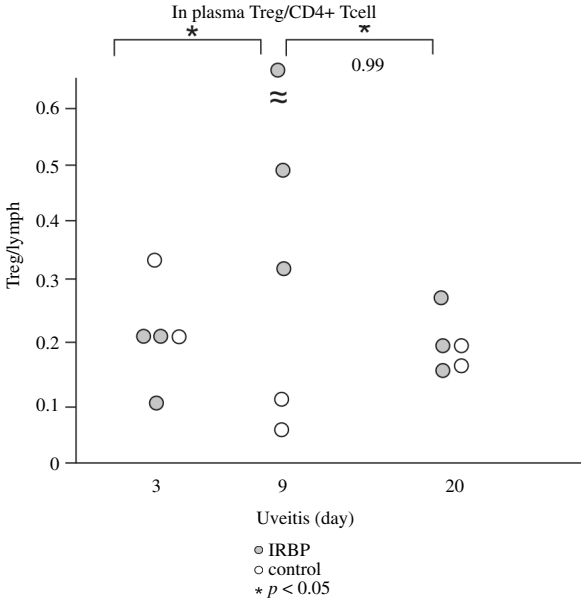

Fig. 3. Treg cells from peripheral blood in mice were measured using two color flow cytometry

lar attack were found to be significantly lower than the normal level [5]. Thus, Treg cells play a pivotal role in ocular attack in BD patients. In addition, percentages of Treg cells in peripheral blood may be a predictive marker of ocular activity in BD patients, facilitating prophylactic treatments of BD patients before ocular attack.

Once the blood-ocular barrier is broken, primed pathogenic T cells migrate into the eyes, followed by the infiltration of other leukocytes, and these cells mediate the immune and inflammatory reactions in the uvea and retina. Histological examinations showed the number of inflammatory cells that had infiltrated the eyes. In immunologically normal mice, EAU is a cell-mediated, Th-1-dependent disease that targets the neural retina where the target antigens are located, leading to the irreversible destruction of photoreceptor cells and loss of vision.

Experimental autoimmune uveoretinitis is controlled by natural Treg cells. Grajewski *et al.* studied whether Treg cells that protect from IRBP-induced EAU require the endogenous expression of IRBP. They showed that IRBP knockout (KO) mice possess EAU-relevant Treg cells that down-regulate responses to IRBP and limit the generation of uveitogenic T cells. Mycobacterial components in completing Freund's adjuvant (CFA) activate Treg cells of other specificities to inhibit the generation of IRBP-specific effector T cells in a bystander fashion, indicating that effective Treg cells can be antigen-nonspecific. Finally, Grajewski *et al.* reported that EAU that is induced in mice with IRBP is controlled by the injection of Treg cells [7].

Terrada *et al.* reported the role of Herpes viruses in the etiology of non-necrotizing retinopathies with typical features of Behçet's disease, birdshot retinochoroidopathy, and idiopathic retinal vasculitis [8]. They developed a new mouse model of uveitis based on the stable retinal expression of influenza virus hemagglutinin (HA) neoantigen by adeno-associated virus-mediated gene transfer. In their mice, the depletion of CD4+CD25+ regulatory T cells exacerbated the disease, whereas the disease could be down-regulated by the administration HA-specific CD4+CD25+ regulatory T cells [8].

As described in previous reports, CD4+CD25+ natural suppressor T cells control uveitis. The depletion of Treg cells exacerbated the disease [9, 10]. Treg deficiency induced by neonatal thymectomy was associated with the development of autoimmune uveitis [10]. In addition, some reports demonstrated the therapeutic potential of Treg cells in various autoimmune diseases including systemic lupus erythematosus (SLE) [11], Sjögren's syndrome [12], rheumatoid arthritis [13-15], and type-1 diabetes [16]. The balance between activated responder T and Treg cells may influence the extent of immunoregulation in human diseases. The number of Treg cells is increased by immunosuppressive treatments that down-regulate inflammation [17]. Tumor necrosis factor α (TNF-α) has a direct effect on Treg cell viability, such as the induction of apoptosis, which would explain the increased number of Treg cells after TNF-α neutralizations [18]. In fact, recently, anti-TNF-α therapy was shown to be useful for treatment in BD patients with uveitis.

In summary, we demonstrated the role of CD4+CD25+ regulatory T cells in EAU mice. Using such models help us clarify the pathophysiology and novel therapy for uveitis.

References

1. Sakane T, Takeno M, Suzuki N, Inaba G (1999): Behçet's disease. N Engl J Med 341: 1284-1291.
2. Nanke Y, Kotake S, Ogasawara K, et al. (2003): Raised plasma adrenomedullin level in Behçet's disease patients. Mod Rheumatol 13: 139-142.
3. Nanke Y, Kotake S, Momohara S, et al. (2002): Synovial histology in three Behçet's disease patients with orthopedic surgery. Clin Exp Rheumatol 20: 35-44.
4. Sakaguchi S, Sakaguchi N, Shimizu J, et al. (2001): Immunological tolerance maintained by CD25+CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 182: 18-32.
5. Nanke Y, Kotake S, Goto M, et al. (2008): Decreased percentages of regulatory T cells in peripheral blood of patients with Behçet's disease before ocular attack: a possible predictive marker of ocular attack. Mod Rheumatol 18: 354-358.
6. Caspi RR, Roberge FG, Chan CC, et al. (1988): A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with tow different retinal antigens. J Immunol 140: 1490-1495.
7. Grajewski RS, Silver PB, Agarwal RK, et al. (2006): Endogenous IRBP can be dispensable for generation of natural CD4+CD25+ regulatory T cells that protect from IRBP-induced retinal autoimmunity. J Exp Med 203: 851-856.

8. Terrada C, Fisson S, De Kozak Y, et al. (2006): Regulatory T cells control uveoretinitis induced by pathogenic Th1 cells reacting to a specific retinal neoantigen. J Immunol 176: 7171-7179.
9. Takeuchi M, Keino H, Kezuka T, et al. (2004): Immune responses to retinal self-antigens in CD25+CD4+ regulatory T-cell-depleted mice. Invest Ophthalmol Vis Sci 45: 1879-1886.
10. Avichezer D, Grajewski RS, Chan CC, et al. (2003): An immunological privileged retinal antigen elicits tolerance: major role for central selection mechanisms. J Exp Med 198: 1665-1676.
11. Crispin JC, Martínez A, Alcocer-Varela J (2003): Quantification of regulatory T cells in patients with systemic lupus erythematosus. J Autoimmun 21: 273-276.
12. Gottenberg JE, Lavie F, Abbed K, et al. (2005): CD4CD25high regulatory T cells are not impaired in patients with primary Sjögren's syndrome. J Autoimmun 24: 235-242.
13. van Amelsfort JM, Jacobs KM, Bijlsma JW, et al. (2004): CD4+CD25+ regulatory T cells in rheumatoid arthritis: difference in the presence, phenotype, and function between peripheral blood and synovial fluid. Arthritis Rheum 50: 2775-2785.
14. Cao D, Malmström V, Baecher-Allan C, et al. (2003): Isolation and functional characterization of regulatory CD25brightCD4 T cells from the target organ of patients with rheumatoid arthritis. Eur J Immunol 33: 215-223.
15. Jiao Z, Wang W, Jia R, et al. (2007): Accumulation of FoxP3-expressing CD4(+)CD25(+) T cells with distinct chemokine receptors in synovial fluid of patients with active rheumatoid arthritis. Scand J Rheumatol 36: 428-433.
16. Kukreja A, Cost G, Marker J, et al. (2002): Multiple immunoregulatory defects in type-1 diabetes. J Clin Invest 109: 131-140.
17. Ehrenstein MR, Evans JG, Singh A, et al. (2004): Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF-α therapy. J Exp Med 200: 277-285.
18. Sarin A, Conan-Cibotti M, Henkart PA (1995): Cytotoxic effect of TNF and lymphotoxin on T lymphoblasts. J Immunol 155: 3716-3718.