의학박사 학위논문

실험적 자가면역 포도막염 병인기전에 미치는 **interleukin-22** 역할에 관한 연구

The role of interleukin (IL)-22 in the pathogenesis of experimental autoimmune uveitis

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서울대학교 대학원

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ABSTRACT

The role of interleukin (IL)-22 in the pathogenesis of experimental autoimmune uveitis

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IL-22 is a pro- and anti-inflammatory cytokine that is mainly produced by T cells and NK cells. Recent studies have reported the increased number of IL-22 producing T cells in patients with autoimmune noninfectious uveitis; however, the correlation between IL-22 and uveitis remains unclear. In this study, I aimed to determine the specific role of IL-22 and its receptor in the pathogenesis of uveitis. Serum concentration of IL-22 was significantly increased in uveitis patients. IL-22Rα was expressed in the retinal pigment epithelial cell line, ARPE-19. To examine the effect of IL-22, ARPE-19 was treated with recombinant IL-22. The proliferation of ARPE-19 and the production of monocyte chemoattractant protein (MCP)-1 from ARPE-19 were clearly elevated. IL-22 induced MCP-1 which facilitated the migration of inflammatory cells. Moreover, IL-22 increased the IL-22Rα expression in ARPE-19 through the activation of PI3K/Akt. Experimental animal models of uveitis induced by interphotoreceptor retinoid binding protein 1-20 ($IRBP_{1-20}$) exhibited elevation of

hyperplasia RPE and IL-22 production. When $CD4^+$ T cells from the uveitis patients were stimulated with $IRBP_{1-20}$, the production of IL-22 definitely increased.

In addition, I examine the regulatory role of cysteamine, which has an anti-inflammatory role in the cornea, in uveitis through the down-regulation of IL-22Rα expression. Cysteamine effectively suppressed the $IRBP_{1-20}$-induced IL-22Rα expression and prevented the development of $IRBP_{1-20}$-induced uveitis in the experimental animal model. These finding suggest that IL-22 and its receptor have a crucial role in the development and pathogenesis of uveitis by facilitating inflammatory cell infiltration, and that cysteamine may be a useful therapeutic drug in treating uveitis by down-regulating IL-22Rα expression in RPE.

Keywords: Experimental autoimmune uveitis, IRBP, Cysteamine, IL-22, Th17, IL-22 receptor

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LIST OF ABBREVIATIONS

ARPE-19: human arising retinal pigment epithelia cell line

Con A: concanavalin A

EAU: experimental autoimmune uveitis

ELISA: enzyme-linked immunosorbent assay

HRP: horse radish peroxidase

IL: interleukin

IRBP: interphotoreceptor retinoid-binding protein

MCP-1: monocyte chemotactic protein-1

NK cell: natural killer cell

pAkt: phosphorylated Akt

PBMCs: peripheral blood mononuclear cells

PBS: phosphate buffered saline

PBST: PBS containing 0.1% Tween 20

PFA: paraformaldehyde

PI3K: phosphoinositide 3-kinase

RORγt: RAR related-orphan receptor gamma

rIL-22: recombinant interleukin-22

Th17: T-helper type 17 cells

INTRODUCTION

Uveitis is an inflammatory disease that develops in uvea which is the middle layer of tissue in the eye wall. It is a very serious disease because of its sudden development and quick progression. Even though it is known that bacterial infection and autoimmune responses are the major cause of disease development, early diagnosis and treatment are the most important in preventing disease progression. Experimental autoimmune uveitis (EAU) is used as an animal model to examine the pathogenesis of uveitis because it represents posterior segment intraocular inflammation in humans. EAU is organ-specific, and it is a T cell-mediated autoimmunity that is induced by immunization with retinal antigens, for example, interphotoreceptor retinoid-binding protein (IRBP) and S-Ag. Additionally, it also can be induced by adoptive transfer of retinal Ag-specific T cells (1-4). In IRBP exposure, inflammatory factors such as Th1/Th17 cytokines increase in the affected tissues, which in turn activate cellular inflammatory responses characterized by infiltration of large numbers of inflammatory cells composed primarily of mononuclear cells (5). Thus, EAU may be a consequence of a Th1/Th17 dominant immune response. Supporting this idea, the numbers of Th17 cells along with elevated levels of particular subsets of IL-17 have been reported to be increased in EAU, suggesting a mechanism by which Th17 cells may contribute to uveitis (6).

Th17 cells have been identified as a subset of T helper lymphocytes characterized by the production of the IL-17 cytokine family, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F (7). It has also been reported that Th17 cells also

produce IL-22 in both mice and human (8). Additionally, it has been newly designated as Th22 (9-11). It is known that epithelial cells are the major target for IL-22. Moreover, it has a crucial role in the wound healing process as well as in controlling bacterial infection (12-15). The IL-22 receptor consists of IL-22Rα and IL-10Rβ. IL-22 exerts its biological effects through binding to the heterodimer IL-22Rα/IL-10Rβ complex followed by activation of signal transducer and activator of transcription 3 (STAT3) (12, 16, 17). It is known that IL-10Rβ is ubiquitously expressed in a variety of cell types. Moreover, the expression of IL-22Rα is restricted to epithelial cells, especially to keratinocytes in the skin and hepatocytes in the liver (12, 16-19). Even though it is not expressed on immune cells, it has recently been reported that it is expressed on $CD11b^+$ APC in mice through stimulation with IRBP (20).

It seems that IL-22 is closely related with autoimmune diseases such as rheumatoid arthritis (RA), Crohn's disease, and skin inflammatory diseases by promoting inflammatory responses (21-23). In contrast, IL-22 is also described as an anti-inflammatory cytokine family because its protective role has been reported in inflammatory bowel disease, experimental hepatitis, and experimental autoimmune myocarditis (24-27). Recent studies have shown that IL-22 gene expression was increased in patients with autoimmune noninfectious uveitis through gene analysis (28). In addition, fresh intraocular T cells from mice with EAU contained a large population of $IL\text{-}22^+$cells, suggesting that Th22 cells may be associated with the pathogenic mechanisms of intraocular inflammation (29-31). However, there are no

studies on the biological features of IL-22 in the pathogenesis of uveitis and much remains to be explored about the role of IL-22 in EAU.

Cysteamine (2-aminoethanthiol) is currently used for the clinical treatment of nephritic cystinosis and used to treat cysteine crystal buildup in the cornea of patients with cystinosis (32, 33). It has a strong antioxidant activity and has been implicated in the treatment of inflammation and neurodegenerative disorders (34-36). In recent studies, cysteamine decreased the proliferation of PBMCs, the secretion of IL-6 and the TGF-β1 levels through ROS formation suggesting it targets inflammation-associated PBMCs that interact with corneal endothelial cells (37). Because IL-6 is one of the inducers of IL-22 production, it suggests that cysteamine could also down-regulate IL-22 production.

Therefore, I examined whether the production of IL-22 and its receptor expression are increased in EAU and their role in the pathogenesis of EAU. Additionally, I investigated the anti-inflammatory effects of cysteamine in a murine model of EAU to determine whether cysteamine has a therapeutic potential effect on patients with uveitis by down-regulating IL-22 and its receptor.

MATERIALS AND METHODS

Sample collection from patients with uveitis The research performed in this study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Seoul National University Hospital (protocol No.1503-027-654). After written informed consent was obtained, samples of serum and PBMCs were collected from 20 patients with a well-defined clinical diagnosis of acute and fresh uveitis without any medication and from 19 healthy donors as normal controls. The subjects were uveitis patients at the Boramae Medical Center and Seoul National University Hospital in Seoul, Korea. The healthy control subjects had no clinical history of uveitis or systemic diseases.

Cell culture Human retinal pigment epithelia cell line (ARPE-19) was obtained from the American Type Culture Collection (ATCC) (Manassas VA, USA) and cultured in DMEM/F12 (WELGENE, Daegu, Korea) with 10% fetal bovine serum (GIBCO, Grand Island NY, USA), 100 U/ml of penicillin and 100 μg/ml of streptomycin (GIBCO) in a humidified incubator at 37℃ and 5% CO_2.

Confocal microscopic analysis ARPE-19 cells grown on coverslips were fixed with 4% paraformaldehyde (PFA) in PBS at 4℃ for overnight and then rinsed three times before use. For the immunocytochemistry staining, the ARPE-19 cells on the coverslips were incubated with 5% goat serum in 0.3% Triton X-100 in PBS at room temperature (RT) for 1 h to exclude nonspecific staining. These cells were then

incubated with rabbit anti-IL-22Rα (1:100; abcam, Cambridge, UK) overnight at 4℃. After washing with PBS for three times, the cells were incubated with Alexa fluor-633 conjugated anti-rabbit antibody (Ab) (1:2,000; Invitrogen, Carlsbad, CA, USA) for 60 min at RT and then DAPI stained. Eye tissues isolated from exprimental mouse were fixed in 4% PFA in PBS at 4℃ overnight. The mouse groups were control, IRBP-induced EAU animals, and IRBP-induced EAU group treated with cysteamine. The fixed eye samples were embedded in paraffin and sectioned. The paraffin sections were stained with rabbit anti-IL-22Rα (1:200; abcam) as primary Ab at 4℃ overnight and Alexa fluor-633 conjugated anti-rabbit Ab (1:2,000; Invitrogen) for 60 min at RT as secondary Ab.

Enzyme-linked immunosorbent assay (ELISA) ARPE-19 cells at 2×10^4 were incubated for 48 h in the presence or absence of rIL-22 (10 ng/ml; R&D Systems). The concentration of TNF-α, IL-6, IL-8 and MCP-1 in the culture supernatants was measured by ELISA kits (BioLegend, San Diego CA, USA). The relative absorbance was measured at 450 nm using a microplate reader and the SoftMax Pro software (Molecular Devices, Sunnyvale CA, USA).

Migration assay A total of 5×10^4 ARPE-19 cells suspended in serum-free media treated with or without rIL-22 (10 ng/ml) were placed in the lower compartment of a transwell migration chamber (Costar, Corning Inc., Corning NY, USA). The upper chamber with a 8 μm-pore transparent PET-filter was then coated with Matrigel Basement membrane matrix (BD Biosciences, San Jose CA, USA) and incubated at

4℃ for 24 h. PBMCs at 1×10^5 were then placed in the upper compartment, and cells were allowed to migrate for 72 h with conditioned media in the lower compartment in the presence or absence of anti-MCP-1 Ab (0.2 μg/ml) (R&D Systems, Minneapolis MN, USA). After 72 h, cells were visualized with Diftquick-staining solution (Sysmex, Chuo-ku, Kobe, Japan).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) To examine the signaling pathways for IL-22Rα expression, ARPE-19 cells at 3×10^5 were pre-treated with a specific inhibitor for PI3K/Akt (LY294002; 10 μM; Sigma-Aldrich, St. Louis MO, USA) for 1 h. After rinsing with PBS, the cells were then cultured for 6 h in rIL-22 (10 ng/ml). The primer used for the RT-PCR was as follows: 5'-CCCCACTGGGACACTTTCTA-3' (forward) and 5'-TGGCCCTTTAGGTACTGTGG-3' (reverse) for IL-22Rα (243 bp); 5'-CATTGGGAATGGTACCAC-3'(forward);and 5'-CCAATAATGGTGTCATCCAC-3' (reverse) for IL-10Rβ (291 bp). To elucidate the correlation between $IRBP_{1-20}$ and the IL-22Rα levels, cells at 3×10^5 were treated with $IRBP_{1-20}$ (1 μg/ml) for 1, 2, 4, and 6 h and collected at the indicated time points. In addition, the ARPE-19 cells were treated with or without $IRBP_{1-20}$ and cysteamine (2.5 μg/ml) for 6 h.

Western blot analysis ARPE-19 cells at 5×10^5 were incubated in the presence or absence of rIL-22 (10 ng/ml) for 5, 10, 20, and 30 min. The cells were then lysed and proteins were extracted using the lysis buffer containing 50 mM Tris-HCl (pH 7.4), 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA in

presence of protease inhibitor cocktails and phosphatase inhibitor cocktails. An equal amount of protein at 25 μg per sample was eletrophoresed in a 12% polyacrylamide-SDS gel at 100 V for 4 h and transferred onto a nitrocellulose membrane. Blocking was performed for 1 h at RT with 5% nonfat milk in PBS containing 0.1% Tween 20. The blocked membrane was incubated with anti-pAkt Ab (1:1,000; Cell signaling Technology, Boston, MA, USA), anti-Akt Ab (1:1,000; Cell signaling technology) or anti-β-actin (1:8,000; Sigma-Aldrich) at 4℃ overnight. After washing for 3 times with PBS, 0.1% Tween 20 (5 min/each), the membrane was incubated with HRP-conjugated anti rabbit IgG (1:15,000; Cell signaling technology) for p-Akt, Akt and HRP-conjugated anti-mouse IgG (1:10,000; Cell signaling technology) for β-actin for 1 h at RT as secondary Ab and washed as before.

[^{3}H]-Thymidine incorporation assay ARPE-19 cells (5×10^3) were seeded in 96-well flat plate with the presence of absence of rIL-22 (5, 10 ng/ml) for 48 h . For inhibitor study, ARPE-19 cells at 5×10^3 were seeded in a 96-well plate with the specific inhibitors ERK (PD98059; 20 μM; Sigma-Aldrich), JNK (SP600125; 20 μM; Sigma-Aldrich), PI3K/Akt (LY294002; 10 μM; Sigma-Aldrich), p38 MAPK (SB203580; 10 μM; Sigma-Aldrich), and NF-κB (Bay11-7082; 5 μM; Sigma-Aldrich) for 1 h. After rinsing with PBS, cells were cultured in the presence or absence of rIL-22 (10 ng/ml). After 30 h, 1 μCi of [^{3}H]-Thymidine (American Radiolabeled Chemicals) was added to each well. After an18 h incubation, cells were harvested onto glass fiber filters using a cell harvester (Inotech Biosystems International). When dry, these were sealed into polyethylene bags with scintillation fluid (BetaplateScint),

and incorporated [^{3}H]-thymidine was counted on a MicroBeta Trilux 1450 (PerkinElmer).

PI/RNase staining ARPE-19 cells at 3×10^{5} were treated with in presence or absence of rIL-22 (10 ng/ml) for 48 h. Cells harvested with trypsin were washed with PBS and fixed with 70% ethanol at 20℃ for a minimum of 2 h. Cells were then washed with PBS to remove the ethanol and centrifuged for 5 min at 1,500 rpm. For staining, the cells were resuspended in 0.5 mL of propidium iodide (PI)/RNase staining buffer (BD Biosciences) at RT for 15 min and analyzed by flow cytometry.

Mice EAU model with IRBP and treatment with cysteamine Eight to ten-week-old female C57BL/6 mice weighing about 20 g each were purchased from Koatech (Pyeongtaek, Gyeonggi, Korea). The animals were handled and cared for according to the Association for Research in Vision and Ophthalmology (ARVO) guidelines for use of animals in ophthalmic and vision research and with the approval of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Seoul, Korea). Human IRBP peptide sequence 1-20 ($IRBP_{1-20}$) (PTHLFQPSLVLDMAKVLLD) was purchased from Peptron (Daejon, Korea). Purified Bordetella pertussis toxin (PTX) and cysteamine were purchased from Sigma-Aldrich, and complete Freund's adjuvant (CFA) and Mycobacterium tuberculosis strain H37Ra were purchased from Difco (Detroit MI, USA). Cysteamine was dissolved in PBS at 2 mg/ml and stored in aliquots at -80℃. To induce EAU, 250 μg of $IRBP_{1-20}$ was emulsified in CFA (1:1 v/v) containing 2.5

mg/ml of M. tuberculosis H37Ra. A total of 100 μl of the emulsion was administered by injection into a footpad. Concurrent with immunization, 0.7 μg of PTX in 300 μl of PBS was injected intraperitoneally as an additional adjuvant.

Cysteamine treatment Starting one day before $IRBP_{1-20}$ injection, cysteamine was administered daily and intraperitoneally at 40 mg/kg for 21 days. Control mice were administered intraperitoneally by injection of the same amount of PBS using the same schedule as the cysteamine-administered group. In the negative control group, neither $IRBP_{1-20}$ nor cysteamine was injected into the mice.

Evaluation of EAU Clinical assessment of the retinal inflammation was made with fundoscopy under a binocular microsope after dilation of the pupil. Fundoscopies were carried out every 3 or 4 days from the day 10 after immunization following inoculation. Eye drops containing tropicamide 0.5% and phenylephrine 0.5% (Mydrin-P, Santen Parmaceutical, Osaka, Japan) were applied to the eyes to dilate the pupil. After confirming mydriasis, the fundus of the eye was examined with a surgical microscope and cover glass. Two ophthalmologists performed the clinical assessments in a masked fashion. The presence of vessel dilatation, white focal lesions, white linear lesions, retinal hemorrhage, and retinal detachment was determined. The severity was graded on a scale of 0 (no disease) to 4 (severe disease) using the clinical criteria as described elsewhere (5). At 21 days after immunization, the mice were euthanized with CO_2 gas. After excision, the eye tissues were processed the tissue for histopathological assessment. The freshly enucleated eyes

were fixed with 4% PFA and were embedded in paraffin. Next, sections of 4-μm thickness were prepared and stained with hematoxylin and eosin (H&E) solution. Histological grading was assessed in a double-blind manner on a scale of 0 (no disease) to 4 (severe disease) as reported. Higher score of the two eyes was adopted as the severity for each mouse.

Flow cytometry analysis Spleen and draining lymph nodes derived from the mice post immunization at day 14 with $IRBP_{1-20}$ were prepared. The spleen and lymph nodes were aseptically removed and placed into as washing media containing cold RPMI containing 10% heat-inactivated feral bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Homogenates were passed through 70 μm nylon mesh and centrifuged at 600 x g for 10 min. The resulting pellet was harvested and resuspended in RBC lysis buffer. After a wash with washing media, the cells were resuspended in FACS buffer containing 0.5% BSA and blocked at 4℃ for 10 min with Fc blocking reagent (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). And then cells were stained with anti- TCR β, NK1.1, CD11c, CD19, CD11b, CD8 and CD4 antibodies on ice for 30 min and washed twice with FACS buffer. The cells were analyzed by FACS caliber (BD Biosciences).

Cytokine expression of T cells in draining lymph node For cytokine analysis, nylon wool-purified T cells derived from mice post immunization at 7 with $IRBP_{1-20}$ were prepared from their draining lymph nodes. Inguinal and popliteal lymph nodes were isolated. Using a plunger from a disposable plastic syringe, I mashed the nodes

against a sterile stainless-steel mesh in a petri dish containing sterile medium. The preparation of single-cell suspension was made with collagenase and DNAse treatment. Cells at 1×10^6 cells were stained with PE-Cy5- or FITC-conjugated Ab specific for anti-mouse CD3 or CD8 (eBioscience, San Diego, CA, USA) at 4℃ for 20 min. The cells were then permeabilized using Cytofix/Cytoperm kit from eBioscience, followed by intracellular staining performed with APC-conjugated Ab specific for IFN-γ and IL-10, and PE-conjugated Ab for IL-17 and IL-4 (eBioscience) according to the manufacturer's instruction. Cells were analyzed using a BD FACSCalibur flow cytometry (BD Biosciences), with a minimum of 10,000 live cells per sample collected and evaluated.

IL-22 levels in splenocytes with EAU and CD4$^+$ T cells from uveitis patients For $IRBP_{1-20}$-specific T cell assay, the $IRBP_{1-20}$-induced EAU mice were injected with cysteamine (40 mg/kg) intraperitoneally for 14 days. After the splenocytes were isolated from the $IRBP_{1-20}$-injected mice treated or untreated with cysteamine, they were re-stimulated with $IRBP_{1-20}$ (1 ug/ml) *in vitro*. After 24 h, the supernatants from the cultures media were collected and assayed by ELISA for the detection of IL-22 levels. PBMCs were isolated from the blood samples of uveitis patients (n=6) and healthy donors (n=6). Using the CD4$^+$ T cells isolation kit (130-096-533, Miltenyi Biotec, Auburn, CA, USA), human CD4$^+$ T helper cells are isolated from PBMCs. The CD4$^+$ T cells were cultured in the presence or absence of $IRBP_{1-20}$ (1 μg/ml) and cysteamine (2.5 μg/ml) *in vitro* for 24 h. The culture supernatants were collected and used for the detection of IL-22 via ELISA.

Surface and intracellular RORγt staining

PBMCs were isolated from blood sample of uveitis patients (n=6) and healthy donors (n=6) and were cultured in the presence or absence of IRBP at 1 μg/ml and cysteamine at 2.5 μg/ml *in vitro* for 24 h. Surface staining was performed at 4℃ for 30 min with anti-CD3 Ab-FITC. Rat IgG Ab was used as an isotype control. After surface staining, the cells were resuspended in Fixation/Permeabilization solution (BD Cytofix/Cytoperm kit, BD Biosciences), and intracellular RAR related-orphan receptor gamma 2 (RORγt) staining was performed according to the manufacturer's protocol. The culture supernatants were collected and used for detection of IL-22 and IL-17 via ELISA.

Statistical analysis

Data are presented as the means ± SEM. Statistical analysis of EAU scoring and marker-positive $CD4^+$ T cell population were done using the Mann-Whitney test and Kruskal-Wallis test with Bonferroni's correction for transglutaminase activity analysis. P values <0.05 were used to indicate a statistically significant difference (GraphPad Software, La Jolla, CA, USA).

Results

1. ***Differential serum IL-22 levels among uveitis patients and healthy donors*** In my study, the serum IL-22 levels in the uveitis patients were significantly increased compared to those in the healthy controls (mean value ± SEM) (uveitis patients, n=20; 63.35 ± 15.36 pg/ml *vs.* healthy controls, n=19; 13.40 ± 3.002 pg/ml; *p=0.0036*) (Figure 1). Gender, age, and disease status are described in Table 1.

	Healthy donors	Uveitis patients
Number	19	20
Age	33 ± 7.9	38 ± 15.5
Sex	Women: 10, Men: 9	Women: 10, Men: 10
Clinical phase	N/A	Acute
Medication	N/A	w/o any medication

(*Age: mean ± SD)

Table 1. Clinical information

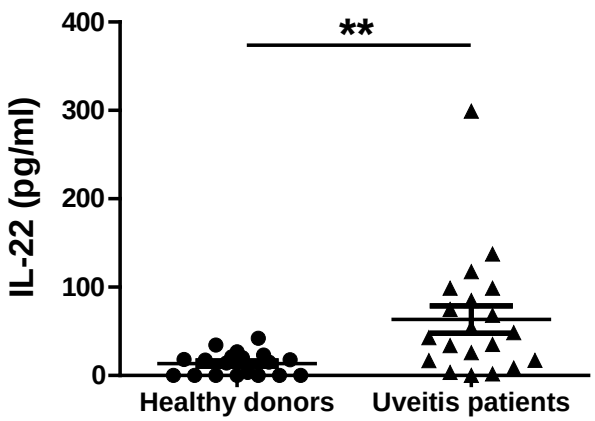

Figure 1. IL-22 serum levels in patients with uveitis.

Serum was obtained from patients with uveitis (n=20) and healthy donors (n=19). It was separated from whole blood using a serum-separating tube by centrifuging at 600 x g for 10 min. IL-22 serum levels were measured with ELISA kits following the manufacturer's instructions. *P-value* was obtained using unpaired two-tailed student's t-test. ** $p<0.001$

2. *Increased MCP-1 in ARPE-19 cells by treatment with rIL-22*

IL-22 exerts its effects through binding to its receptors on target cells, although, it is not yet known which are these cells and how they function within the eye in a uveitis setting involving the posterior segment. Therefore, RT-PCR and confocal microscopy were used to detect IL-22Rα expression in ARPE-19 cells. From RT-PCR analysis, the mRNA of IL-22Rα was expressed in ARPE-19 cells (Figure 2A). From the confocal microscope pictures, the expression of IL-22Rα was distributed throughout the cellular membrane and in the cytoplasm but not in the nucleus (Figure 2B). There was no staining in the secondary antibody only as a control.

To determine the effect of IL-22 on the production of inflammatory cytokines by ARPE-19 cells, the cells were cultured with rIL-22 (10 ng/ml) for 24 h. In the presence of rIL-22, ARPE-19 cells produced significantly higher amounts of MCP-1 than those cultured in the control medium (control; 904.2 ± 99.61 pg/ml *vs.* rIL-22-treated; 2347 ± 190.4 pg/ml; *p=0.0026*). However, the rIL-22-treated ARPE-19 cells produced similar levels of TNF-α, IL-6 and IL-8 as those of the control cells (Figure 3).

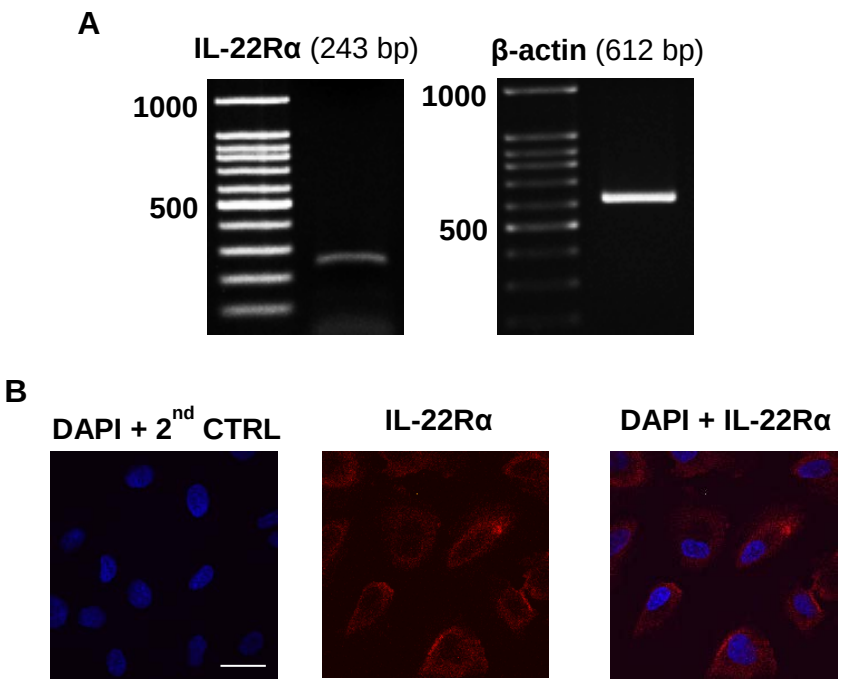

Figure 2. The expression of IL-22Rα in human arising retinal pigment epithelia cell line, ARPE-19

(A) 1.5% agarose gel electrophoresis of PCR product amplified by the IL-22Rα and IL-10Rβ primers. (B) Expression of IL-22Rα in ARPE-19 cells was analyzed by confocal microscopy. Scale bar = 20 μm

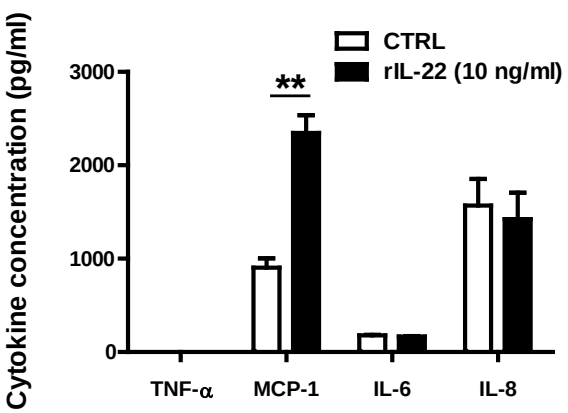

Figure 3. Production of pro-inflammatory cytokines in ARPE-19 cells by treatment with rIL-22

ARPE-19 cells were incubated with rIL-22 at 10 ng/ml for 24 h and the concentration of TNF-α, MCP-1, IL-6 and IL-8 was measured with ELISA. Results are representative of three independent experiments. **p<0.001

3. ***Migration of peripheral blood mononuclear cells in response to IL-22***

To elaborate on the previous results, migration of PBMCs in response to rIL-22-treated ARPE-19 cells was measured using a migration assay. PBMCs at the upper chamber migrated to the basement of trans-well with ARPE-19 cells with or without rIL-22 in the bottom chamber and were visualized by microscopy (Figure 4). Since blocking of MCP-1 using anti-MCP-1 Ab inhibited the migration of the PBMCs, it suggested that MCP-1 produced by ARPE-19 cells treated with rIL-22 drove the migration of PBMCs such as monocytes and lymphocytes. The results indicate that IL-22 may induce the increased production of MCP-1 leading to recruited macrophages into the retina. According to the report by Murao K et al, PI3K/Akt pathway plays a crucial role in the production from vascular endothelial cells by TNF-α stimulation (38). Therefore, an inhibitor study was carried out using LY294002, a potent PI3K inhibitor. As shown in Figure 5, MCP-1 production from ARPE-19 was increased by rIL-22 treatment, but it was inhibited by pre-treatment of LY294002 (control treated with LY294002; 1738 ± 27.69 pg/ml *vs.* rIL-22 treated with LY294002; 1566 ± 92.81 pg/ml; $p=0.1503$). It suggests that PI3K/Akt also plays a role in the production of MCP-1 by rIL-22 stimulation.

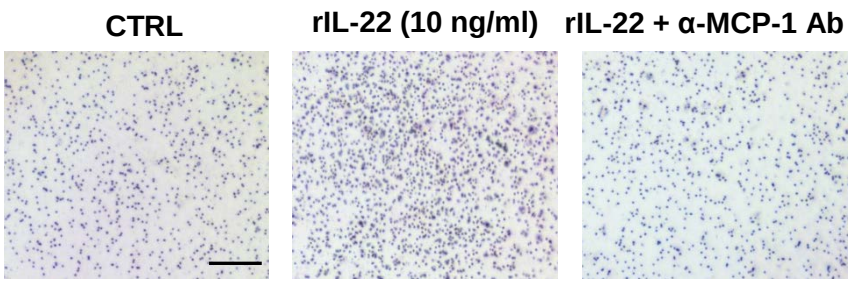

Figure 4. Migration of PBMCs in response to MCP-1 produced by ARPE-19 cells treated with rIL-22

Migration of PBMCs in response to IL-22 was measured by the migration assay. ARPE-19 cells were placed in the lower chamber with ARPE-19 cells with or without rIL-22 and the upper chamber with PBMCs. PBMCs were then allowed to migrate for 72 h. Anti-MCP-1 Ab at 0.2 μg/ml was used for blocking MCP-1. Data were visualized with Diftquick-staining solution. Scale bar = 200 μm

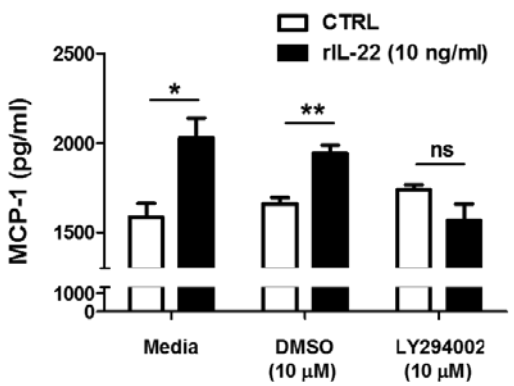

Figure 5. PI3K/Akt dependent production of MCP-1 in ARPE-19 cells treated with rIL-22

ARPE-19 cells were pre-treated with DMSO (vehicle control) and LY294002 at 10 μM for 1 h and then cultured with rIL-22 at 10 ng/ml for another 24 h. Results are representative of three independent experiments. * $p<0.05$, ** $p<0.001$

4. Positive feedback amplification of IL-22Ra in ARPE-19 cells

IL-22 receptor complex leads to the activation of JAK1 and Tyk2 kinases, followed by activation of the STAT-3, and often STAT-1 and/or STAT-5. In addition to JAK/STAT3 signaling, IL-22 activates the three major MAP kinase pathways of JNK, p38 MAPK, and ERK-1/2 (9, 39). Although IL-22 exerts its effect mainly though activation of STAT-1, STAT-3, STAT-5 along with activation ERK-1/2 and p38 MAPK, a recent study has shown that IL-22 can also activate important kinases such as PI3K/Akt. As shown in Figure 5, PI3K inhibition blocked the secretion of MCP-1 and thus, PI3K/Akt signaling pathway may be involved in MCP-1 production. Since IL-22 led to secretion of MCP-1, I speculated that IL-22 might lead to activation of Akt. This hypothesis bore out as Figure 6, phospho-Akt expression was increased after 10 min by the stimulation with 10 ng/ml of rIL-22.

Because a chronic relapsing uveitis is induced by autoreactive T cell subsets which are producing pro-inflammatory cytokines, I investigated that whether IL-22 could lead to an increase of expression of it receptors in a positive feedback loop and possibly potentiating the effect of IL-22 in development of uveitis. To this end, I performed an RT-PCR using specific primers against IL-22Rα and IL-10Rβ. In Figure 7, IL-22 in ARPE-19 led to an increased expression of IL-22Rα possibly via activation of PI3K/Akt pathway and phosphorylation of Akt. Since there was no change for levels of IL-10Rβ, it suggested that IL-22 led to a positive feedback mediated through the increased expression of IL-22Rα.

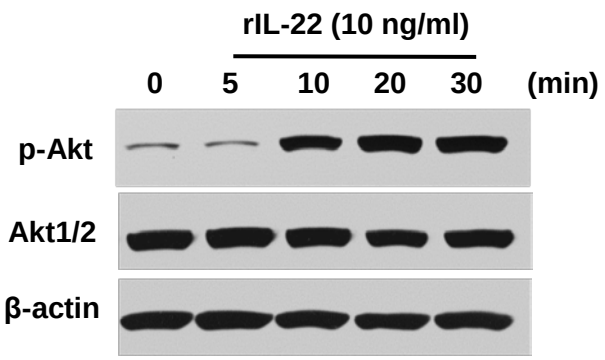

Figure 6. Increase of the phosphorylation of Akt in ARPE-19 cells treated with rIL-22

ARPE-19 cells were treated with rIL-22 at 10 ng/ml, and western blot for phosphorylation of Akt and Akt1/2 was performed as described in the Materials and Methods.

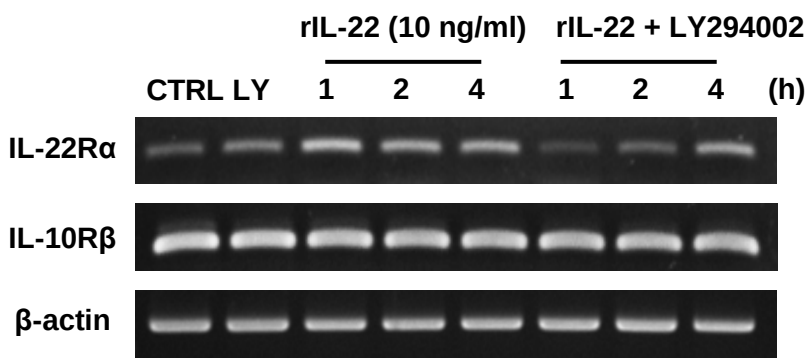

Figure 7. The effect of IL-22 on ARPE-19 cells through the regulation of its receptor expression

For mRNA expression, ARPE-19 cells were treated with rIL-22 at 10 ng/ml alone or combined with LY294002 at 10 μM, an inhibitor of PI3K, and collected at the indicated time points, followed by RT-PCR.

5. Increased proliferation of ARPE-19 cells treated with rIL-22

IL-22 induces the proliferation of normal human epidermal keratinocytes obtained from healthy individuals and fibroblast like synoviocytes isolated from psoriatic arthritis, rheumatoid arthritis and osteoarthritis patients (22, 40-42). In a thymidine incorporation assay, recombinant IL-22 at 10 ng/ml induced a dose-dependent proliferation of ARPE-19 cells (control, 1592 ± 133.9 cpm; rIL-22 at 5 ng/ml, 2397 ± 228 cpm; and rIL-22 at 10 ng/ml, 3213 ± 263.3 cpm) (control *vs.* rIL-22 at 5 ng/ml, *p=0.0251*)(control *vs.* rIL-22 at 10 ng/ml, *p=0.0008*) (Figure 8). In my study, ARPE-19 cells were pre-treated with the specific inhibitors for ERK (PD98059), JNK (SP600125), PI3K/Akt (LY294002), p38 MAPK (SB203580), and NF-κB (Bay11-7082) for 1 h. When ARPE-19 cells were treated with rIL-22, I could observe p38MAPK and NK-κB dependent proliferation of ARPE-19 cells (Figure 9).

Furthermore, to determine whether the cell cycle phase was attributable to proliferation, PI/RNase staining was performed. FACS analysis revealed that with exposure to rIL-22 at 10 ng/ml for 48 h, more cells accumulated more cells in the G2/M phase of the cell cycle. Quantitatively, 26.2% of IL-22 treated cells as opposed to 19.8% of control cells were in the G2/M phase (Figure 10).

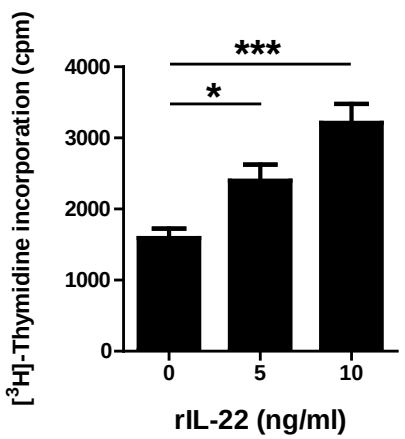

Figure 8. The proliferation of ARPE-19 cells by the treatment of rIL-22

ARPE-19 cells were treated with 10 ng/ml of rIL-22 for 48 h. For the last 18 h, 1 μCi of [^{3}H]-thymidine was added to per well. Results are representative of three independent experiments. .* $p<0.05$ ***, $p<0.0001$

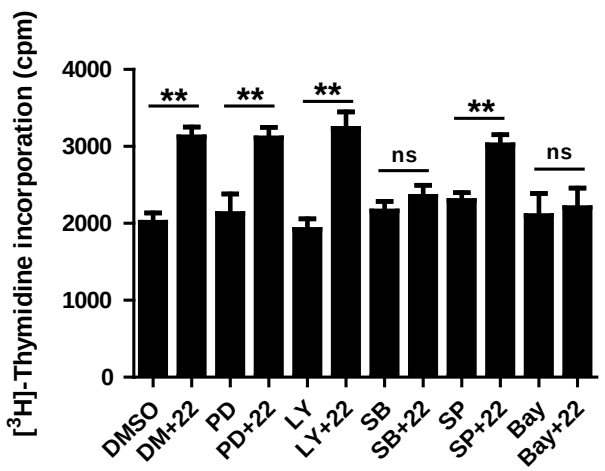

Figure 9. P38 MAPK and NK-κB dependent proliferation of ARPE-19 cells treated with rIL-22

Cells were pre-treated with DMSO (vehicle control), SB203580 (20 μM), SP600125 (20 μM), PD98059 (20 μM), LY294002 (10 μM) and Bay11-7082 (5 μM) for 1 h and then treated with rIL-22. After culturing for another 48 h, proliferation of ARPE-19 cells was measured as described in Materials and Methods. *P-value* was obtained using unpaired two-tailed student's t-test. ** $p<0.001$

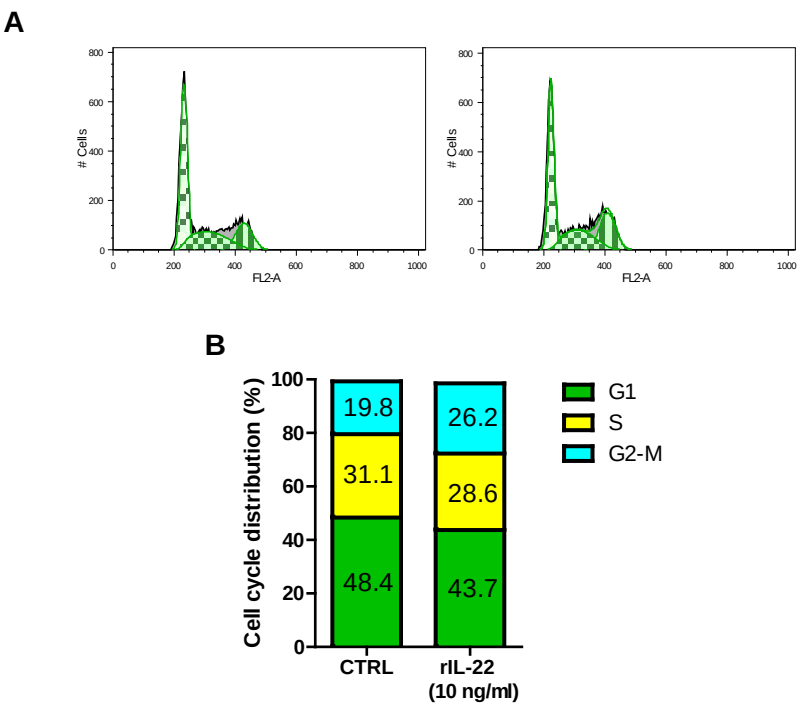

Figure 10. Cell cycle analysis in APRE-19 cells treated with rIL-22

ARPE-19 cells were treated with 10 ng/ml of rIL-22 for 48 h. (A) ARPE-19 cells were analyzed by flow cytometry after staining with PI/RNase. (B) The bar graph shows that values are the percentage of cells in each cell cycle phase.

6. Attenuation of the severity in EAU by treatment of cysteamine

Next, I investigated the potential anti-inflammatory effect of cysteamine in the IRBP-induced EAU in C57BL/6 mice. Clinical grading was carried out every 3 or 4 days from 10 days after immunization. Clinical examinations were performed in the control (PBS treated) and cysteamine treated (40 mg/kg) groups. In the control group (n=21), vascular sheathing and multiple yellow dots were evident in the fundus at the 13 days. For the cysteamine-treated group (n=18), the fundoscopic examination disclosed mild vascular sheathing at 13 and 21 days post immunization (Control *vs.* cysteamine; at 13 days, $p=0.01$; and at 21 days, $p= 0.02$) (Figure 11A). Disease onset was significantly delayed in cysteamine-treated group compared with that in control group (control; 12.1 ± 1.4 days *vs.* cysteamine; 14.6 ± 3.0; $p=0.02$, Mann-Whitney test). The histological score in mice treated with cysteamine was also significantly lower than that in control mice (control *vs.* cysteamine; $p=0.03$) (Figure 11B). Light microscopy of ocular sections showed normal-appearing retina and there was no systemic toxicity. Representative histopathology of the eyes from mice treated with cysteamine (n=27) or PBS (n=26) is shown in Figure 12.

Inflammatory cells along with retinal folds and granulomatous lesions were more severe in the retina, vitreous and choroid of control mice compared to the cysteamine-treated mice at 21 days post immunization.

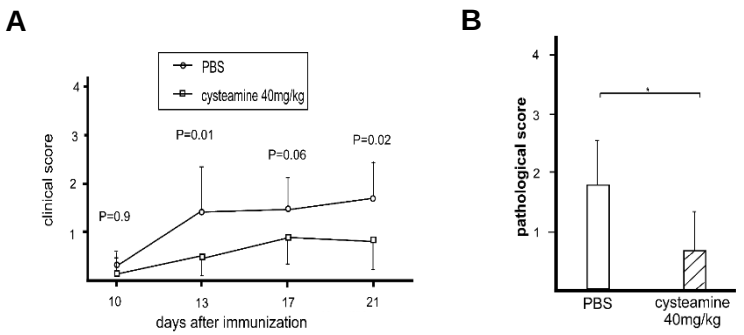

Figure 11. The effect of cysteamine on EAU

(A) A diagram of clinical scoring of IRBP-induced uveitis. Clinical score of EAU in mice treated with vehicle (PBS, n=21) alone or cysteamine (40 mg/kg) (n=18). EAU was induced as described in Materials and Methods. Results are presented as the mean clinical score for all eyes for each group of mice and significance was determined using the Mann-Whitney test. (B) A diagram of histopathology scores of IRBP-induced uveitis. Histopathological score of EAU was determined by histopathology on day 21 post immunization. Each symbol represents one mouse, showing the higher score of two eyes for each mouse. Mean EAU score of each group is indicated by a bar. The pathological scores of retinal sections were significantly lower in cysteamine-treated group (n=27) than in control group (n=26). Results are expressed as mean ± standard deviation and significance was determined using the Mann-Whitney test. * $p<0.05$

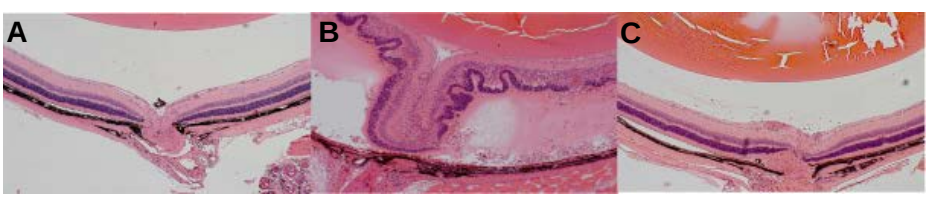

Figure 12. The effect of cysteamine on EAU

Representative histopathology of mice treated with vehicle (PBS) alone or cysteamine. Negative control (A), mouse treated with PBS (B), and mouse treated with cysteamine (C). On day 21 post immunization, the eyes from normal and EAU mice were enucleated and scored by examining the histopathological sections of these eyes (H&E, x100 magnification).

7. Inhibition of the Th17 cytokines treated with cysteamine

To examine whether cysteamine treatment could alter Th1/Th2/Th17 population in the splenocytes of treated animals, I quantified IFN-γ, IL-4, IL-10 and IL-17 levels by intracellular FACS staining. The expression of IL-17 in $CD4^+$ T cells of cysteamine-treated group was decreased compared to those in PBS-treated group at day 7 post immunization ($p=0.03$, Mann-Whitney test) (Figure 13). The expression of IFN-γ was also lower in cysteamine-treated group than in control group, but it only had a borderline significance ($p=0.06$). In addition, the expression of IL-4 and IL-10 was not different between PBS- and cysteamine-treated groups ($p>0.05$). However, there was no difference of population between PBS-and cysteamine-treated groups (Figure 14-15).

IRBP-induced EAU is associated with the release of pro-inflammatory cytokines such as IFN-γ and IL-17, which are key mediators in the induction, course, and severity of this model. Thus, the levels of pro-inflammatory cytokines IFN-γ, TNF-α IL-2, IL-4, IL-6, IL-10, IL-17 and IL-22 in plasma were analyzed. Since Th17 cells are characterized as a novel $CD4^+$ subset of T cells that preferentially produce IL-22, the effect of cysteamine on the production of IL-22 was examined. I injected cysteamine (40 mg/kg) intraperitoneally into the IRBP-induced EAU mice for 14 days. After splenocytes were isolated from IRBP-injected mice treated or untreated with cysteamine, they were re-stimulated with IRBP at 1 ug/ml for 24 h in vitro at which time the supernatants from the cultures were collected and assayed for cytokines. The level of IL-22 by ELIA was significantly increased in IRBP-treated

splenocytes compared to control-treated cells (Figure 16). However, cells that were treated *in vivo* with cysteamine showed a significantly decreased production of IL-22 compared with those that were not treated with cysteamine.

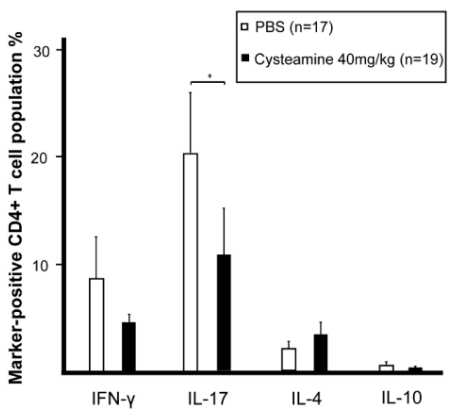

Figure 13. Intracellular cytokine staining of IFN-γ, IL-4, IL-10 and IL-17 in $CD3^+CD4^+$ T cells

After gating for $CD3^+CD4^+$ T cell population, the samples were stained with anti-IL17, IFN-γ, IL-4, and IL-10 antibodies. Results are expressed as mean ± standard deviation and significance was determined using the Mann-Whitney test. * $p<0.05$

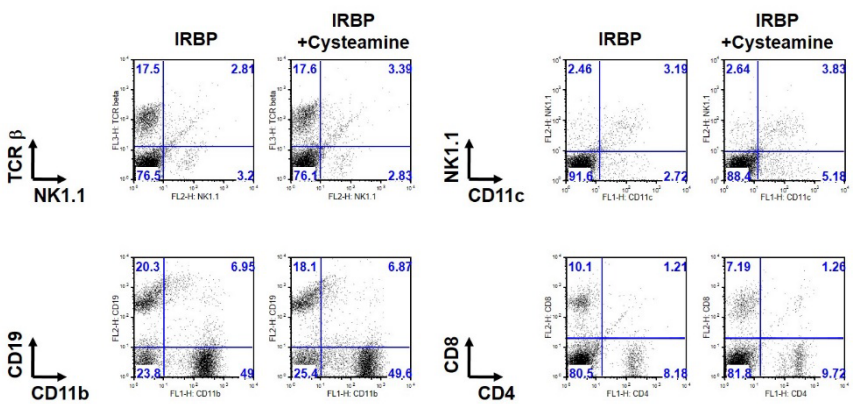

Figure 14. The population of NK-T, NKDC, B cells and T cells from splenocytes

Population of NK-T, NKDC, B cells and T cells from splenocytes was determined by flow cytometry at day 14. The splenocytes were isolated and stained as described in Materials and Methods.

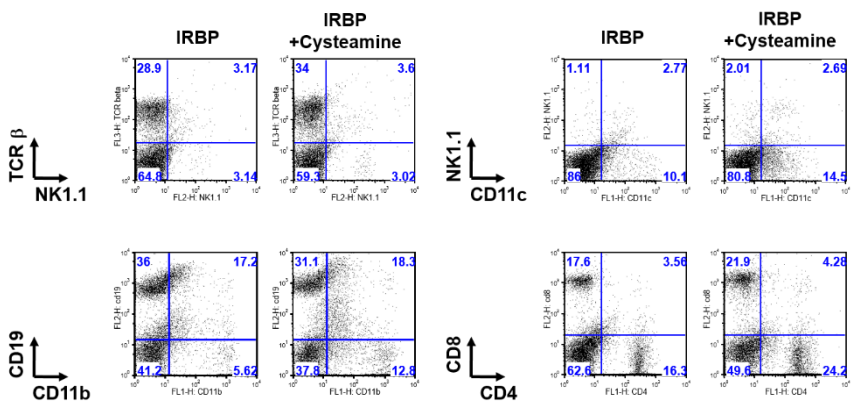

Figure 15. The population of NK-T, NKDC, B cells and T cells from lymph nodes

Population of NK-T, NKDC, B cells and T cells from lymph nodes was determined by flow cytometry at day 14. The lymph nodes were isolated and stained as described in Materials and Methods.

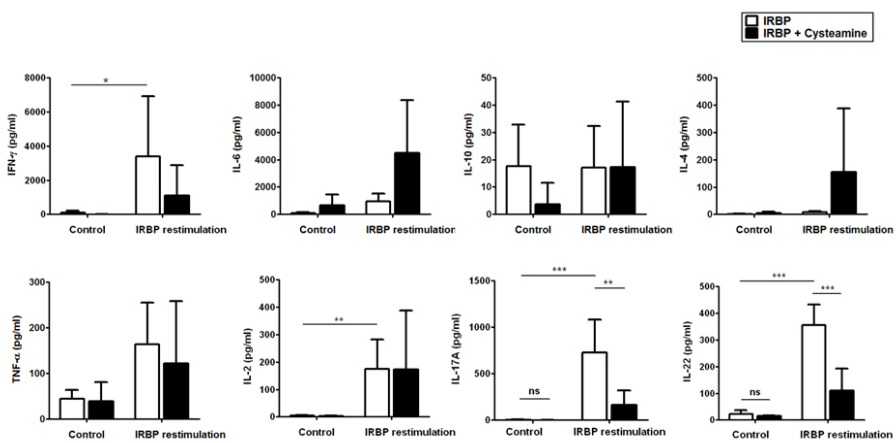

Figure 16. Production of Th1, Th2 and Th17 cytokines from splenocytes

Splenocytes from IRBP-treated and IRBP-treated with cysteamine injected mice were re-stimulated with IRBP at 1 μg/ml *in vitro* for 24 h. The supernatants from the cultures were collected and assayed for CBA and ELISA. *P-value* was obtained using unpaired two-tailed student's t-test. *** $p<0.0001$

8. Down-regulation of RORγt expression and IL-22 production by cysteamine

To investigate that whether cysteamine could regulate the Th17 cell differentiation and IL-22 production, I isolated PBMCs from uveitis patients and healthy donors. In Figure 17, the RORγt expression was increased by IRBP in $CD4^+$ T cells from uveitis patients and healthy donors in $CD4^+$ T cells treated with IRBP and cysteamine. In addition, cysteamine treated cells had lower IL-22 levels for PBMCs treated with IRBP in uveitis patients (Figure 18). These results show that cysteamine controls the differentiation of $ROR\gamma t^+$ T cells with uveitis patients.

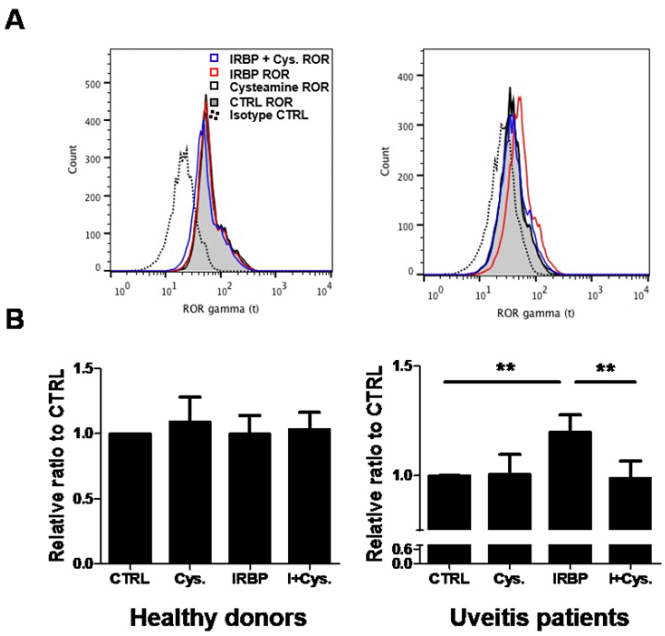

Figure 17. Comparison of RORγt expression in the peripheral CD4$^+$ T cells of uveitis patients (n=6) and healthy donors (n=6)

PBMCs were isolated from the blood of uveitis patients and healthy donors. And then, the cells were cultured in presence or absence of IRBP at 1 μg/ml and cysteamine at 2.5 μg/ml *in vitro* for 24 h. Intracellular RAR related-orphan receptor gamma (RORγt) staining was performed as the manufacturer's protocol. (A) Representative histogram data from one of six independent experiments. (B) Relative ratio was mean fluorescence intensity (M.F.I.) relative to each control. *P-value* was obtained using unpaired two-tailed student's t-test. ** $p<0.001$

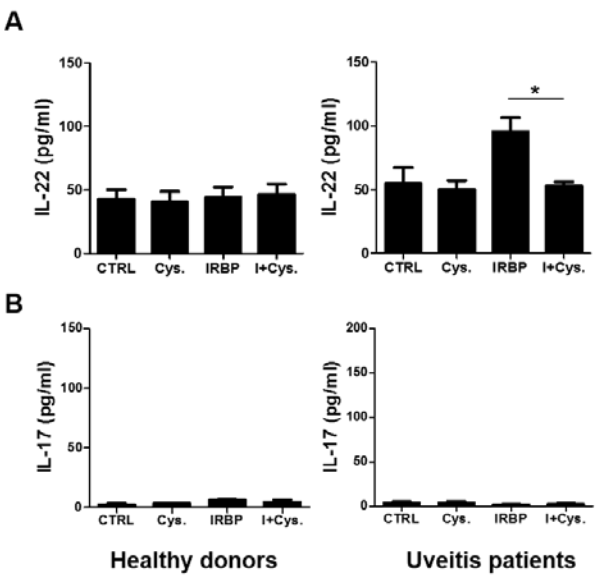

Figure 18. Comparison of IL-22 production from the peripheral CD4$^+$ T cells of uveitis patients (n=6) and healthy donors (n=6)

IL-22 levels in the PBMCs of uveitis patients (n=6) and healthy control (n=6). *P-value* was obtained using unpaired two-tailed student's t-test. * $p<0.05$

9. Down-regulation of IL-22Rα by cysteamine *in vitro* and *in vivo*

Because IL-22Rα expression was shown to be upregulated in the liver from concanavalin A injected mice in a previous study (25), I investigated whether IRBP and cysteamine regulated the expression of IL-22Rα in ARPE-19 cells. Cells were treated with IRBP at 1 μg/ml for 1, 2, 4, and 6 h and collected for RT-PCR analysis. IRBP upregulated IL-22Rα expression in ARPE-19 cells in a time-dependent manner (Figure 19A). In addition, the upregulated IL-22Rα induced by IRBP in ARPE-19 cells was downregulated by the treatment of cysteamine (Figure 19B).

To histologically examine the response of IRBP and cysteamine, eye tissues were isolated from each group of mice: control mice, IRBP-induced EAU mice, and IRBP-induced EAU mice treated with cysteamine. IRBP upregulated IL-22Rα expression in retinal pigmented epithelium and induced retinal detachment. However, I observed the downregulation of IL-22Rα expression in eye from cysteamine-treated mice. Therefore, the effect of cysteamine on ARPE-19 cells observed earlier may be through a downregulation of IL-22Rα levels (Figure 20).

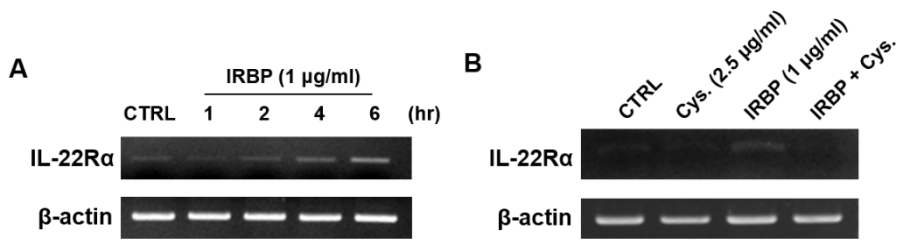

Figure 19. The regulation of IL-22Rα expression in ARPE-19 cells treated with cysteamine

(A) Cells were treated with the IRBP at 1 μg/ml for 1, 2, 4, and 6 h and collected for RT-PCR analysis at the indicated time points. (B) Cells were cultured with or without IRBP and cysteamine at 2.5 μg/ml for 6 h. RT-PCR was performed as described in Materials and Methods.

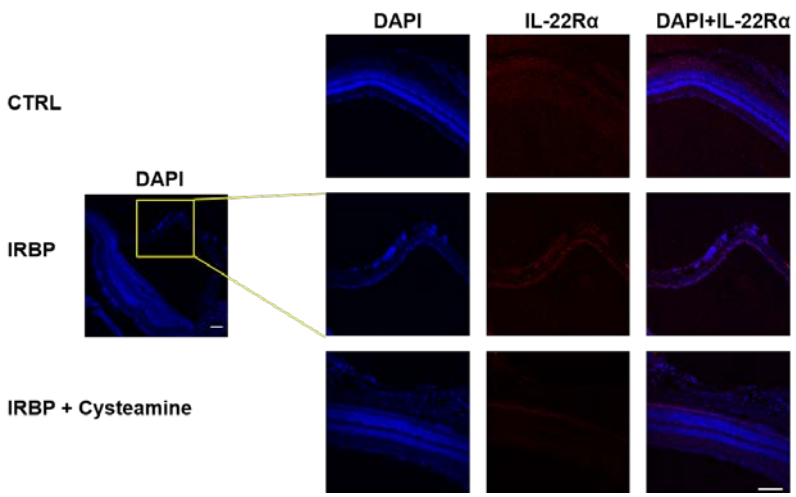

Figure 20. The regulation of IL-22Rα expression in eye treated with cysteamine

Eye tissues isolated from each mouse (control *vs.* IRBP-induced EAU model *vs.* IRBP-induced EAU model treated with cysteamine) were fixed in 4% PFA at 4℃ overnight. The fixed eye were embedded in paraffin and sectioned. The expression of IL-22Rα in eye tissue was assessed by confocal microscopy. Scale bar = 100 μm

DISCUSSION

Because uveitis is a clinically heterogeneous group of disorders in which intraocular inflammation can lead to visual loss and blindness, accurate diagnosis and successful treatment remains a challenge for the clinicians. Furthermore, many of the severe and chronic types of uveitis require long-term therapy (43). Despite aggressive immunosuppressive therapy (44), in some cases as mentioned above, vision is lost. Therefore, there is an unmet medical need for novel therapies to improve the long-term visual prognosis of uveitis patients.

It is well-known that Th17 cells exacerbate pathogenesis of uveitis (45, 46), so I can assume that IL-22 produced by Th17 cells, Th22 cells, natural killer cells, and dendritic cells may have a role in various inflammatory scenarios in uveitis (9, 29). In this study, I evaluated the biological effect of IL-22 and the therapeutic effect of cysteamine in EAU. IL-22 serum levels were higher in patients with uveitis than those in healthy donors (Figure 1). IL-22 acts via a heterodimeric receptor complex that consists of the IL-22Rα subunit and the IL-10Rβ subunit. IL-10Rβ is ubiquitously expressed in various cell types including immune cells, but neither resting, nor activated immune cells express IL-22Rα. However, IL-22Rα expression is restricted to nonhematopoietic cells, such as the cells in the skin, pancreas, intestine, liver, lung and kidney (9). Also, IL-22Rα is also expressed in retinal pigment epithelial cell line, ARPE-19 cells (Figure 2). IL-22 exerts its biologic functions through IL-22R and I showed that in ARPE-19 cells, IL-22 is part of a positive feedback loop on these cells where IL-22 leads to an increased expression

of IL-22R (Figure 7). This result may be related to the chronic and relapsing nature of uveitis.

The retinal pigment epithelium (RPE) is monolayer of cells situated between the neuroretina and choroid. RPE cells are able to contribute to either immunosuppressive or inflammatory responses in the eye through the secretion of cytokines, antagonists, and soluble cytokine receptors. Of interest, IL-6, IL-8, TNF-α, and MCP-1 are secreted to choroidal side of the RPE layer in a polarized fashion (47-49). Polarized secretion of these cytokines has an important role in immune processes in the posterior part of the eye. Inflammatory cell infiltration in the eye and secretion of inflammatory cytokines lead to intraocular inflammation that can ultimately cause blindness (50). Pathologically, macrophages play a crucial role in the initial phase of uveitis (51, 52). Recruited macrophages can exert their inductive functions on the development and persistence of inflammation through the production of NO and the phagocytosis of rod cells and cone cells in the retina. This aggravates inflammation of posterior segment in the eye (53). In addition, IL-22 from Th17 T cells may play an important role in the inflammatory scenario of uveitis as IL-22 also induces MCP-1 production in autoimmune settings such as rheumatoid arthritis (22). MCP-1 gene transcription is upregulated in response to TNF-α in vascular endothelial cells through the PI3K/Akt (38). As shown in Figure 3-5, IL-22 treatment induced the production of MCP-1 by ARPE-19 cells and its production was downregulated by LY294002. Therefore, RPE may direct lymphocyte and monocyte migration during posterior uveitis via CC chemokines.

Although corticosteroids are effective in reducing eye inflammation, they are associated with a wide range of complications. Since oxidation stress plays a role in development of autoimmune disease in animal models, I explored the anti-oxidant effects of cysteamine (54). As, to my knowledge, there has been no study on whether cysteamine exerts a potent anti-inflammatory on the autoimmune uveitis disease. Externally applied cysteamine reduces inflammation in the eye and had already been used systemically for patients with nephropathic cystinosis (32, 33). In my study, clinical and pathological scores were significantly lower in cysteamine-treated group than in control group (Figure 5). The possible therapeutic mechanism of cysteamine in inflammation could be via elimination of reactive oxygen species (ROS) (55). Because ROS clearly play key roles in modulation T cell activation and differentiation, inhibition of their activity may be necessary for a variety of autoimmune diseases (56, 57). In addition, superoxide radicals are involved in the pathogenesis of experimental endotoxin-induced uveitis since their detoxification by ebselen, an antioxidant molecule, prevents the disease in both rabbits and rats (58, 59).

In my study, cysteamine was administered to mice with EAU *in vivo* to examine if cysteamine mitigates ocular inflammation with inhibition of Th17 cytokines including IL-22. I showed that there was an anti-inflammatory effect of cysteamine and that it correlated with inhibition of Th17 population and reduced IL-22 production (Figure 13, 16). My data also suggested that abated Th17-cytokine responses might contribute to the reduced severity EAU. The precise molecular

mechanism by which cysteamine participating in the inhibition of ocular inflammation needs to be further studied.

RORγt is expressed exclusively in cells of the immune systems such as $CD4^+CD8^+$ double-positive thymocytes, LTi, LTi like cells, Th17, and Th22. RORγt is a nuclear receptor with a ligand binding pocket and the transcription factor that directs the differentiation of inflammatory Th17 cells and the production of their cytokines (60, 61). In case of experimental autoimmune encephalomyelitis (EAE), RORγt is a key regulator of immune homeostasis with a potential as a therapeutic target in inflammatory disease (62). In my study, I found that RORγt expression is related to the constitutive production of IL-22 in T cells with uveitis patients. I demonstrated that cysteamine through the down-regulation of RORγt possibly inhibits the differentiation of Th17 cells through the down-regulation of RORγt and its cytokine production *in vitro*. (Figure 17-18). My data support the premise that suppression of effector T cell differentiation by cysteamine therapy may ameliorate severe ocular inflammation in uveitis patients.

Vitamin C, another strong antioxidant, demonstrated a protective effect in an in vivo model of liver damage through the maintenance of IL-22Rα signaling pathways (25). Similarly, cysteamine also modulated the expression of IL-22Rα in ARPE-19 and IRBP-induced EAU mice (Figure 19-20).

Given that IL-22 is a pro-inflammatory cytokine in uveitis, an important question is whether IL-22 can be a therapeutic target. In uveitis, a variety of cytokines, including MCP-1, TNF-α and IL-6, are thought to contribute to tissue

damage. This has been proven by the fact that regulating these cytokines is beneficial in patients with uveitis. There are several limitations in my study. I did not demonstrate the mechanism of Th17 suppression. Also it is not known whether amelioration of EAU by cysteamine could be attributed to the inhibition of IL-22 production by $CD4^+$ T cells or by another mechanism such as down-regulation of the ability of dendritic cells to prime T cells toward the Th17 effector pathway. Further work is required for the mechanism of depressed immunological responses. Additionally, I did not measure the level of intraocular IL-22. To retrieve the aqueous humor from the eyes in mice was difficult and inconsistent. However, I discovered a close relationship between the level of IL-22 from splenocytes and inflammatory status in the development of EAU and the modulation by cysteamine led to an improvement in clinical parameters with the corresponding histological changes.

In this study, the effects of cysteamine as a treatment of endogenous uveitis and a regulator of IL-22 production and its receptor were to be investigated. I suggest that cysteamine, which is an approved drug without significant side effect, may be tested for treatment of ocular inflammation. Since uveitis is characterized by being persistent and recurrent, besides being an unmet medical need therapeutically, it can also be considered a social and economic burden for the affected individuals. If an effective therapy is found, the economic benefit can also be considered to be very large

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국 문 초 록

실험적 자가면역 포도막염 병인기전에 미치는 interleukin-22 역할에 관한 연구

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IL-22는 염증유발 혹은 항 염증 역할을 하는 사이토카인으로써 주로 T세포와 자연살해세포에서 분비된다. 최근 연구결과에 따르면, IL-22를 분비하는 T세포가 자가면역 비감염성 포도막염 환자에서 증가되어 있으나, 아직까지 그 관계에 대해선 불명확하다고 알려져 있다. 본 연구에서는 포도막염 발병에 있어서 IL-22와 그 수용체의 역할에 대해 알아보고자 한다. 그 결과, 포도막염 환자의 혈청 내 IL-22 농도가 정상인에 비하여 증가되어 있었으며, 사람 망막 색소 상피 세포주인 ARPE-19에서 IL-22 수용체 α 발현을 확인하였다. 다음으로, IL-22의 역할을 규명하기 위하여, ARPE-19 세포주에 재조합 된 IL-22을 처리하였다. IL-22는 ARPE-19 세포주의 증식과 MCP-1의 분비를 증가시켰으며, IL-22로부터 유도 생산된 MCP-1는 염증 세포들의 이동을 촉진시켰다. 게다가, IL-22는 ARPE-19에서 PI3K/Akt의 활성을 통하여 자기 수용체 발현을 증가시켰다. $IRBP_{1-20}$로 유발한 실험적 자가면역 포도막염 동물모델에서는 망막 색소 상피세포층과 IL-22 생산의 증가가 관찰되었다. 포도막염 환자로부터 분리한 $CD4^+$ T세포에 $IRBP_{1-20}$로 자극을 시켜주면, IL-22의 분비가

현저히 증가된 것을 볼 수 있었다. 게다가, 각막에서 항 염증 역할을 하는 시스테아민이 포도막염에서 IL-22 수용체α발현을 조절 할 수 있는지 알아보고자 하였다. 시스테아민은 $IRBP_{1-20}$로 유발한 실험적 자가면역 포도막염의 동물모델에서 IL-22 수용체α발현과 안구의 염증반응을 효과적으로 억제하였다. 이러한 결과들은 IL-22와 그 수용체가 포도막염의 진행과 발병에 있어서 염증세포의 침윤 촉진을 통해 중요한 역할을 하며, 시스테아민은 망막 색소 상피의 IL-22 수용체α발현을 억제함으로써 포도막염의 효과적인 치료 약물로써의 가능성을 시사한다.

주요어: 실험적 자가면역 포도막염, IRBP, 시스테아민, IL-22, Th17, IL-22 수용체

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